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Article · June 2022

DOI: 10.18488/72.v6i1.3033

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International Journal of Advances in Life Science and Technology
2022 Vol. 6, No. 1, pp. 24-30.
ISSN(e): 2313-8335
ISSN(p): 2412-3803
DOI: 10.18488/72.v6i1.3033

ANTIBACTERIAL ACTIVITIES OF *PENTACLETHRA MACROPHYLLA* AND *SYZYGIUM SAMARANGENSE* AGAINST OPPORTUNISTIC BACTERIA PATHOGENS

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Article History
Received: 14 April 2022
Revised: 18 May 2022
Accepted: 2 June 2022
Published: 23 June 2022

Keywords
P. macrophylla
S. samarangense
Phytochemical
Antibacterial.

ABSTRACT

In this study, *Pentaclethra macrophylla* and *Syzygium samarangense* leaves was tested for their antibacterial activities against selected opportunistic bacterial strains. The crude extracts of the plants were extracted using methanol and fractionated into hexane, ethyl acetate and aqueous fractions. The antibacterial activity of the leaves against *B. subtilis* (ATCC® 6633™), *S. aureus* (ATCC® 25923™), *E. coli* (ATCC® 25922™), *P. aeruginosa* (ATCC® 9027™) was performed using the agar well diffusion technique at 20mg/ml extracts fractions. All plants extracts extract exhibited some antibacterial activity against at least one bacterial strain. *P. macrophylla* exhibited the highest inhibition against *E. coli* (25mm) compared to *B. subtilis*, *S. aureus* and *P. aeruginosa*. Similar observation was noted for *S. samarangense* where the highest inhibition was against *E. coli* (22mm). In conclusion, *P. macrophylla* and *S. samaragense* exhibited antibacterial activities against the selected bacteria. The ethyl acetate and aqueous extracts of both plants had the highest antibacterial activities. This indicates that *P. macrophylla* and *S. samrangense* leaves could be a source of antibacterial agent in overcoming antibacterial drug resistance.

Contribution/Originality: This study presents the phytochemical and antibacterial properties of the methanol, hexane, ethyl acetate and aqueous extracts of the leaves of *Pentaclethra macropylla* and *Syzygium samarangense*. This comparative evaluation in a published paper is scarce and is thoroughly investigated in this paper.

1. INTRODUCTION

Opportunistic microorganisms are microorganisms that only infect immunocompetent individuals in specific situations. They are causal agents of infections in immunosuppressed individuals. The indiscriminate use of antibiotics inhibits the growth of the natural microbiota in the body and increases the risk of infections from extraneous microorganisms which could develop susceptibility to antibiotics [1]. This resistance could be attributed to mutations in drug related targets and acquired genes from other bacterial species [2].

Pentaclethra macrophylla also called the African oil bean belongs to the family Fabaceae [3]. It is commonly found in west African countries [4]. It is an herbal plant with all its parts (bark, seeds, fruits, and leaves) used for

therapeutic purposes in South-eastern Nigeria [5]. Studies have shown that *P. macrophylla* leaves possesses antifungal, antinoceptive, and anti-inflammatory properties [6-8].

S. samarangense (Blume) Merrill is a deciduous tree, also known as Samarang apple, Wax Apple or African Apple. First discovered in Malacca, it has been cultivated in several countries [9]. Diverse papers have reported the medicinal properties of *S. samarangense* for the treatment of several diseases and it is associated with free radical scavenging, antioxidation and anticancer activities [9, 10].

Due to the increasing trend in antibiotic resistance and the severe consequences especially in immunocompromised individuals, drug development and modification is necessary. This study provides an insight into the this activities.

This current study evaluated methanol, hexane, ethyl acetate and aqueous extracts of two medicinal plants (*P. macrophylla* and *S. samaragense*) for their antibacterial activities against selected bacterial pathogens of some selected plants already used in traditional medicine, as possible sources of therapeutic agents.

2. MATERIALS AND METHODS

2.1. Plant Samples Preparation

Pentaclethra macrophylla and *Syzygium samarangense* were selected based on their use in traditional medicine. The plants were authenticated by Dr. Popoola at the Biology programme in the Department of Biological Sciences, Covenant University. The plant leaves were selected for extraction, air at dried at room temperature for 14 days and consequently grounded using Kenwood blender (Model: 09773) into coarse powder.

2.2. Plant Samples Extraction

Plant extraction was performed as documented by Olugbuyiro, et al. [11]. Five hundred (500) g powder of each tested plant material was soaked in 2500 ml litres of methanol and left to macerate for 7 days at room temperature. Decantation of the methanol was performed, and subsequent filtration was done using a Whatman No. 1 filter paper. Continuous extraction was performed using fractionation method to yield hexane, ethyl acetate and aqueous extracts. The extracts were then concentrated using a rotary evaporator (EYELA N-1100, China). The concentrated extracts were reconstituted using Dimethylsulfoxide (DMSO) to obtain a concentration of 20.0 mg/ml and was stored at 4^0C prior to determination of the antibiotic mean susceptibility.

2.3. Preliminary Phytochemical Investigations

Preliminary phytochemical analysis was determined as described by Olugbuyiro [12].

2.3.1. Test for Tannins

Dissolve crude extract (0.5g) in 10 ml of methanol. One millilitre of the original solution should be diluted to 5ml with distilled water. One drop of 10% ferric chloride solution should be added and left for 10 minutes. Formation of blue-black precipitate indicates the presence of tannins.

2.3.2. Test for Phenols

To 1ml of the original solution+1 M NaOH, then added dilute HCL. Observe for color change.

2.3.3. Test for Flavonoids

(a) OLU reagent: to 5 ml of alcoholic solution add 2-3 drops of Olu reagent. The appearance of orange, pink, red or reddish color would indicate the presence of flavonoids.

2.3.4. Test for Saponins

(I) Frothing test:1ml of the dissolved sample was diluted to 5ml with water and then shaken vigorously for 2mins. Frothing was observed for over 15 mins. Persistent frothing was indicative of the presence of saponins.

2.3.5. Test for Anthraquinones

(I) Free anthraquinones: about 0.5g of powdered/original sample was shaken with 5 ml of chloroform for 10 mins and filtered. Then the filtrate was then shaken with 5ml of 10% NH3 solution. Presence of a pink color in the ammonia phase was considered positive.

2.3.6. Test for Alkaloids

The extract solution was acidified with 3 ml of 10% HCl. To 1ml of the filtrate few drops of each of the following reagents were added:

(I) Mayer's reagent.

(II) Dragendorff's reagent.

(III) Wagner's reagent.

Precipitation or turbidity with any of these reagents was taken as preliminary evidence for the presence of alkaloids in the sample.

2.4. Identification of Bacterial Strains

The bacterial isolates were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos; *B. subtilis* (ATCC®6633™), *S. aureus* (ATCC®25923™), *E. coli* (ATCC®25922™), *P. aeruginosa* (ATCC®9027™).

2.5. Antibiotic Susceptibility Testing of Bacterial Isolates

Agar well diffusion technique was used in determining the antibacterial activities of the plant extracts as described by Obinna, et al. [13]. Using a sterile loop, three to four isolated colonies of the test organisms was inoculated individually into 4mL peptone broth. The turbidity of the peptone broth was adjusted to 0.5 McFarland turbidity standard, and the bacteria strains were plated individually on nutrient agar. Thereafter, a cork borer of diameter 6mm was used to make wells on the seeded plates. 20 millilitres (20 ml) of the methanolic, hexanolic, ethyl acetate and aqueous extracts were dispensed into the different wells. Ciprofloxacin was used as the positive control while methanol was used as the negative control. The plates were left at room temperature for one hour to allow the extracts to diffuse into the medium. Using a sterile forceps, the ciprofloxacin antibiotic disc was placed onto the surface of a streaked agar plate. Thereafter, the plates were incubated at 37 °C for 18–24 h and the zones of inhibition were measured.

2.6. Minimal Inhibitory Concentration and Minimal Bactericidal Concentrations

For this study, the minimal inhibitory concentration was defined as the lowest concentration of a drug substance that can inhibit the growth of the bacterial isolates and the minimum concentration that had a bactericidal effect was calculated as the minimal bactericidal concentration [14]. The ethyl acetate and aqueous extracts of both plants extract were prepared using double dilution with DMSO as a diluent to obtain the following concentrations: 20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml. and 1.25 mg/ml. The extracts were introduced into the wells on the seeded plates and incubated at 37°C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimal inhibitory concentration (MIC). The minimal bactericidal concentration (MBC) had similar setup with the MIC but its focus was based on the bactericidal effect of the extract rather than its inhibitory effect.

2.7. Statistical Analysis

The data collected in the study were entered into MS Excel for analysis and storage.

3. RESULTS

3.1. Phytochemical Screening

Phytochemical screening of the methanolic extracts of the leaves of the two plants showed the presence of tannins, saponins, flavonoids, alkaloids, anthraquinones and phenols Table 1.

Table 1. The Phytochemical Profile of the Methanolic Extracts of *P. macrophylla* and *S. samarangense*.

Plants Extract	Tannins	Saponins	Flavonoids	Alkaloids	Anthraquinones	Phenols
Methanolic extracts of *Pentaclethra macrophylla*	++	+++	++	+++	-	++
Methanolic extracts of *Syzygium samarangense*	+++	+++	+++	++	+	+++

Note: (+): level of detection, (-): non-detection or absence.

3.2. Mean Susceptibility Testing

Pentaclethra macrophylla and *Syzygium samarangense* leaves were observed to have varying antibacterial effects against the bacterial isolates Table 2. For *P. macrophylla*, high antibacterial activity was observed for the ethyl acetate and aqueous fractions ranging from 16-25mm, and 11-20mm respectively. For the ethyl acetate assay, it showed the mean zone of inhibition of 25mm for *E. coli* (ATCC®25922™) and 21mm for *B. subtilis* (ATCC®6633™). The methanol and hexane extracts had low zones of inhibition of 12-17mm and 1-5mm respectively. Also, ethyl acetate extract of *S. samaragense* showed the mean zone of inhibition of 22mm for *E. coli* (ATCC® 25922™) and 20mm for *B. subtilis* (ATCC® 6633™). The aqueous extracts of *S. samarangense* also showed moderate activity with a range of 12-20mm similar to the aqueous extracts of *P. macrophylla*. Also, the methanol and hexane extracts of *S. samarangense* had low zones of inhibition ranging from 6-13mm and 5-12 mm respectively. Ciprofloxacin had significant results against *B. subtilis*, *S. aureus* and *P. aeruginosa* but had a lesser antibacterial activity on. *E. coli.*

Table 2. The antibacterial activity of the extracts (Zones of Inhibition, mm).

Bacterial Test Strains	*Pentaclethra macrophylla* (mm)				*Syzygium samarangense* (mm)				Ciprofloxacin (mm)	Methanol (mm)
	ME	HE	EAE	AE	ME	HE	EAE	AE	Positive Control	Negative Control
B. subtilis (ATCC®6633™)	15	4	21	11	13	10	20	16	20	-
S. aureus (ATCC®25923™)	12	2	16	17	6	5	16	12	21	-
P. aeruginosa (ATCC®9027™)	17	1	17	16	9	6	19	17	21	-
E. coli (ATCC®25922™)	17	5	25	20	12	12	22	20	16	-

Note: ME: Methanolic Extracts, HE: Hexanolic Extracts, EAE: Ethyl acetate Extracts, AE: Aqueous Extracts.

The standard for sensitivity was set at 20mm. Thus, aqueous and ethyl acetate extracts were selected for MIC and MBC.

3.3. Minimum Inhibitory Concentration and Minimal Bactericidal Concentration

Table 3 presents the Ethyl ethyl acetate fraction of *P. macrophylla* had with an MIC of 9mm and *S. samarangense* 2mm on *B. subtilis*. The MBC recorded was 12mm and 11mm respectively. The ethyl acetate MIC on *E. coli* was 9mm for *P. macrophylla* and 9mm for *S. samarangense* and the MBC recorded was 12mm and 11mm respectively.

Likewise, the aqueous fraction of *P. macrophylla* had a MIC of 10mm and 2mm for *S. samarangense* on *B. subtilis*. The MBC recorded was 11mm and 5mm respectively. The aqueous MIC on *E. coli* was 10mm for *P. macrophylla* and 6mm for *S. samarangense* and the MBC recorded was 12mm and 7mm respectively.

Table 3. Minimum Inhibitory Concentration and Minimal Bactericidal Concentration of the ethyl acetate extracts.

Bacterial Test Strains	***Pentaclethra macrophylla*** **(mm)**				***Syzygium samarangense*** **(mm)**			
	Ethyl Acetate Extract		**Aqueous Extracts**		**Ethyl Acetate Extract**		**Aqueous Extract**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
B. subtilis (ATCC®6633™)	5	10	10	11	2	11	2	5
E. coli (ATCC®25922™)	5	10	10	12	9	11	6	7

4. DISCUSSION

This study evaluated the methanol, hexane, ethyl acetate and aqueous extracts of these two medicinal plants for their antibacterial activities against the selected bacterial isolates. The preliminary phytochemical screening of the methanolic extracts of the leaves of *P. macrophylla* and *S. samarangense* revealed the presence of tannins, saponins, flavonoids, alkaloids, anthraquinones and phenols, with the exception of anthraquinones absent in *P. macrophylla*. Ogbonna, et al. [5] reported of similar composition when they studied *P. macrophylla* leaves. However, in the study they found phytate. Also, Osabor, et al. [15] reported of alkaloids, flavonoids, polyphenols and anthranoids in petroleum extract of *P. macrophylla* leaves. Our study revealed slightly different phytochemicals from other studies which indicates that the recoveries of phytochemicals could be affected by their solvent extraction processes. Also, other phytochemicals such as cardiac glycosides were not detected in the methanolic extract used for preliminary phytochemical screening. The antimicrobial susceptibility studies indicated that *E. coli* (ATCC® 25922™) showed resistance to methanol (negative control). The *E. coli* was susceptible to aqueous and ethyl acetate extracts of *P. macrophylla* (20mm and 25 mm) and *S. samarangense* (20mm and 22mm). This observation is similar to the reports of Akah, et al. [16] and Olaitan, et al. [3]. In this study, high zones of inhibition were recorded for *E. coli* using ethyl acetate extracts of leaves of both plants which suggests their effectiveness when used in traditional medicine in confronting ailments implicated by *E. coli*. *P. aeruginosa* (ATCC®9027™) showed resistance to all the extracts and negative control, but was susceptible to Ciprofloxacin. This is similar to results obtained by Ratnam and Raju [10] which showed *P. aeruginosa* resistant to petroleum ether, ethyl acetate and methanol extracts of *S. samarangense* leaves. Also, Khandaker, et al. [9] reported low levels of inhibition of methanol and ethanol extracts of *S. samarangense* leaves against *P. aeruginosa*. The resistance of *P. aeruginosa* to out extracts could be due to the presence of drug resistant plasmids present in the organism. More studies can be done to determine the exact cause of the resistance. *S. aureus* (ATCC® 25923™) exhibited resistance to all extracts of both plants but was susceptible to Ciprofloxacin. Nwakaeze, et al. [17] also reported a low antibacterial activity of cold-water extracts of *P. macrophylla* on *S. aureus*. The cause of resistance of *S. aureus* to the plant extracts could be the emergence of resistant strains via hospital infections, antibiotic abuse and plasmid exchange.

From our study, *B. subtilis* (ATCC® 6633™) was sensitive to ethyl acetate extracts of *P. macrophylla* and *S. samarangense* and Ciprofloxacin. This is similar to results obtained by Chinaka, et al. [18] that reported ethyl acetate fractions of *P. macrophylla* leaves having the highest zone of inhibition on *B. subtilis* compared to other fractions. There seems to be limited studies on the antibacterial activity of *S. samarangense* leaves on *B. subtilis*.

5. CONCLUSION

This study has provided scientific evidence that the ethyl acetate and aqueous extracts of *P. macrophylla* and *S. samarangense* exhibits antibacterial activities against *B. subtilis* (ATCC®6633™), *S. aureus* (ATCC®25923™), *E. coli* (ATCC®25922™), *P. aeruginosa* (ATCC®9027™). Plant leaves are a potential source of useful drugs. Further

studies are encouraged in order to characterize the bioactive compounds present in the plant leaves. This study has laid credence to the uses of the plants for medicinal purposes in traditional medicine practice in Nigeria.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: OSO is a student and was responsible for carrying out the antibacterial analysis alongside the preparation of the manuscript. NO contributed to the experimental design and proofreading. OMI contributed to the antimicrobial analysis, financial support and proofreading. OJ contributed to the experimental design and phytochemical analysis. TO contributed to the experimental design and antibacterial analysis.
Acknowledgement: The authors are grateful to the Department of Microbiology, Covenant University for providing some of the laboratory materials used in this study.

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