American-Eurasian J. Agric. & Environ. Sci., 16 (4): 836-841, 2016
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2016.16.4.10437

Antibacterial Study of Flavonoid Extract of *Peltophorum pterocarpum* Stem Bark

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Abstract: The present study was designed to evaluate the antibacterial activity of methanol and flavonoid extracts of *Peltophorum pterocarpum* stem bark. The plant extract was prepared by maceration in methanol. Extraction of its flavonoids as well as quantitative phytochemical analysis was carried out using standard laboratory procedures. The antibacterial activity assay was performed, using the agar-well diffusion method against pathogenic strains of *Escherichia coli*, *Salmonella typhimurium* Staphylococcus *aureus* and *Bacillus cereus.* Phytochemical analyses of the plant revealed considerable presence of important bio-constituents such as flavonoids, alkaloids, saponins, tannins and phenols. Chloramphenicol (the reference antibiotic drug) showed a concentration- dependent increase in antibacterial activity against the gram- negative and gram- positive bacteria tested. The crude methanol extract as well as the flavonoid constituents also showed concentration- dependent increases in antibacterial activity against the test organisms, inhibiting the isolates with diameter zones of inhibition ranging from 9.0-35.0 mm at concentrations ranging from 6.2-100 mg/ml. The study demonstrated broad spectrum antibacterial activities of the methanol extract, free flavonoids and bound flavonoids against both gram positive and gram negative bacteria. The findings from this study suggested that *P. pterocarpum* stem bark possesses potentials for developing novel antibiotics.

Key words: Antibacterial Activity · Phytochemicals · Free Flavonoids · Bound Flavonoids · Gram-Positive And Gram-Negative Bacteria

INTRODUCTION

Infections caused by multidrug-resistant Gram-positive and Gram-negative bacteria represent a major public health problem, not just in terms of morbidity and mortality, but also in terms of management and implementation of infection control measures [1]. In addition, the development of new antimicrobial drugs must remain a high priority for the continued effective treatment of infections caused by resistant strains [2]. This has necessitated a search for new antimicrobial agents from other sources such as plants; as synthetic drugs are known to cause side effects [3]. The diverse range of phytochemicals found in plants with proven biological activities have continued to attract the attention of both traditional and orthodox medical practitioners [4, 5]. Medicinal plants are useful and economically viable source of antimicrobial agents as they are rich in phytochemicals such as tannins, alkaloids and flavonoids, which have been found *in vitro* to possess antimicrobial properties [6].

Peltophorum pterocarpum belongs to the family *Leguminosae* native to tropical south eastern Asia and a popularly ornamental tree widely distributed around the world including India and Nigeria. It is a deciduous tree growing to 15-25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m. The leaves are bi-pinnate, 30-60 cm long, with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25 mm long and 4-10 mm broad. The flowers are yellow, 2.5-4 cm diameter, produced in large compound racemes up to 20 cm long. The fruit is a pod 5-10 cm long and 2.5 cm broad, red at first, ripening black and containing one to four seeds [7].

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Different parts of this tree are used to treat many diseases such as stomatitis, insomnia, skin troubles, constipation, ringworm and malaria [8, 9]. In Southeast Nigeria, the stem bark is used in the management of malaria and bacterial infections.

Previous studies have shown that roots of *P. pterocarpum* possessed antioxidant and anti-haemolytic activities [6]. Sukurumara *et al.* [10] showed that the aqueous fraction of *P. pterocarpum* flowers exhibited good antibacterial and antifungal activities. This study was designed to investigate the antimicrobial properties of methanol and flavonoid extracts of *P. Pterocarpum* stem bark.

MATERIALS AND METHODS

Collection and Identification of Plant Samples: Fresh stem barks of *Peltophorum pterocarpum* were collected from the Department of Health and Physical Education, University of Nigeria, Nsukka. The plant was identified by Mr. A. Ozioko of Bio resources Development and Conservation Programme Research Centre, Nsukka, Enugu, Nigeria.

Preparation of Plant Material: Fresh stem barks of *P. pterocarpum* were hand-picked from possible contaminants and unhealthy stem barks. They were sliced into smaller pieces, shade-dried for seven days and ground into fine powder using electric grinder and stored in clean-dried container for extraction.

Extraction of *P. pterocarpum* Stem Barks

Crude Extraction Using Methanol: A quantity, 100g of finely ground sample of the *P. pterocarpum* stem bark was extracted with 300ml of methanol using a Soxhlet apparatus for 72 h according to the method described by Sukumaran *et al.* [10]. The extract obtained was concentrated using a rotary evaporator at 40°C. The percentage yield was calculated and the concentrated extract was stored in refrigerator at 4°C until used.

Flavonoid Extraction: The flavonoid content of the *P. pterocarpum* stem bark was extracted using the method of [13]. A known quantity, 100 g of the finely powdered sample was extracted using Soxhlet apparatus with 80% methanol (300 ml) for 24 h. The extract obtained was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analysed for free and bound flavonoid respectively. The ethyl acetate fraction of each of the samples was hydrolysed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract was washed with distilled water to neutrality. Ethyl ether fraction (free flavonoid) was dried in open air and concentrated. Ethyl acetate fractions (bound flavonoid) was dried in the oven at 40°C, weighed and stored in a desiccator.

Test Microorganisms: Clinical isolates of bacterial pathogens used for this work were collected from the Department of Microbiology, University of Nigeria, Nsukka; which were two Gram- negative bacteria, *Escherichia coli* and *Salmonella typhimurium* and two Gram- positive bacteria *Staphylococcus aureus* and *Bacillus cereus*. The bacteria isolates were further purified by sub culturing each isolate into fresh plates of nutrient agar. The pure isolates were identified using standard biochemical methods [12].

Antimicrobial Activity Assay: Agar- well diffusion assay was carried out according to [13]. A volume of 20ml of sterilized agar was poured into sterile Petri plates. After solidification, 100ml of microbial inoculums were swabbed on the respective plates. The wells were punched over their agar plates using sterile gel puncher. The punched agars were filled with 100ml of respective plant extracts at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml in each case). The plates were incubated at 37°C for 24 hours. Chloramphenicol (0.05%) was used as positive control and analysis was done in triplicates. Normal saline was used as negative control.The antibacterial activity was assayed by measuring the diameter of inhibition zone (IZ) formed around the well.

RESULTS

The extraction of 100 g of dry *P. pterocarpum* stem bark with methanol gave a percentage (%) yield of 17.8 % (17.8 g) while the extraction of free and bound flavonoids gave percentage yield of 6.5 and 4.3% respectively.

The data in Table 1 reveals the richness of *P. pterocarpum* stem bark in important phytochemicals with flavonoids as the major phytochemical constituent followed by reducing sugars, alkaloids, terpenoids and carbohydrates in decreasing order, while steroids were the least phytochemical constituent.

Table 1: Quantitative Phytochemical Constituents of *P. pterocarum* Stem Bark

Phytochemical Constituents	Mean Concentrations (mg/100 ml)
Alkaloids	47.69 ± 2.86
Total phenolics	8.13 ± 0.02
Saponins	0.20 ± 0.00
Carbohydrates	22.64 ± 0.15
Terpenoids	31.37 ± 0.03
Flavonoids	73.25 ± 3.70
Tannins	4.26 ± 0.01
Steroids	0.06 ± 0.02
Reducing sugars	60.12 ± 0.32

Values are mean ± standard deviation of the triplicate determination (n = 3)

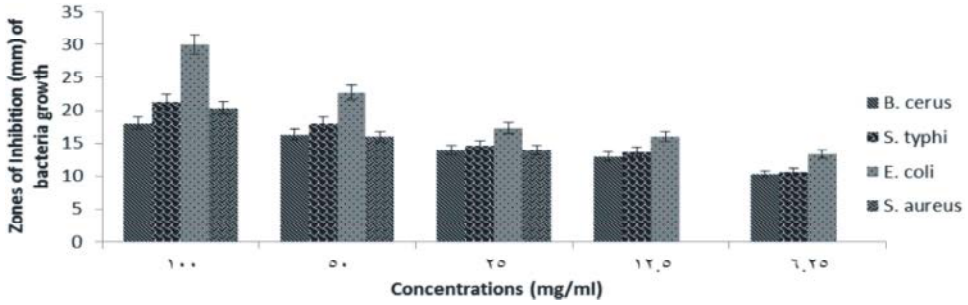

Fig. 1: Zones of Inhibition of Bacterial Growth by the standard antibacterial drug (Chloramphenicol)

Antibacterial Activity Studies: The observation in Figure 1 shows a concentration- dependent antibacterial activity of chloramphenicol (standard drug) against some bacteria in which *E. coli* had highest zones of inhibition at all the test concentrations while *B. cereus* had least zones of inhibition. *S. aureus* exhibited zero zones of inhibition at lower concentrations of chloramphenicol (12.5 and 6.25 mg/ml).

The findings in Table 2 show that *S. typhi* had the highest zone of inhibition followed by *E. coli*, *B. cereus* and *S. aureus* in decreasing order at 100 mg/ml. At 50 mg/ml, *E. coli* had the highest zone of inhibition while *S. aureus* had the least zone of inhibition. However, at concentration of 25 mg/ml, only *E. coli* had zone of inhibition, but at 12.5 and 6.25 mg/ml none of the organisms showed any zones of inhibition.

Table 2: Zones of Inhibition (mm) of Bacteria Growth by the Methanol Extract of *Peltophorum pterocarpum S*tem Bark

Concentration of methanol extract (mg\ml)	Negative Control	B. cereus	S. typhi	E. coli	S. aureus
100	-ve	15.00 ± 1.00	16.67 ± 2.08	15.33 ± 0.58	10.67 ± 0.58
50		12.00 ± 1.00	12.00 ± 1.00	13.67 ± 1.53	9.33 ± 1.53
25		Nil	Nil	11.00 ± 1.00	Nil
12.5		Nil	Nil	Nil	Nil
6.25		Nil	Nil	Nil	Nil

Values are presented as mean ± standard deviation of triplicate determination, nil = zero

Table 3: Zones of Inhibition (mm) of Bacteria by Free Flavonoid of *Peltophorum pterocarpum* Stem Bark

Concentration bound flavonoid extract (mg\ml)	Negative Control	B. cereus	S. typhi	E. coli	S. aureus
100	-ve	12.33 ± 1.73	13.67 ± 1.53	18.00 ± 1.00	12.67 ± 1.53
50		Nil	Nil	14.67 ± 1.53	11.33 ± 0.58
25		Nil	Nil	Nil	Nil
12.5		Nil	Nil	Nil	Nil
6.25		Nil	Nil	Nil	Nil

Values are presented as mean ± standard deviation of triplicate determination, nil = zero

Table 4: Zones of Inhibition (mm) of Bacteria by Bound Flavonoid of *Peltophorum pterocarpum* Stem Bark

Conc. (mg\ml)	Negative Control	B. cereus	S. typhi	E. coli	S. aureus
100	-ve	13.67 ± 1.15	12.00 ± 1.73	13.33 ± 1.53	14.33 ± 2.08
50		Nil	8.33 ± 0.55	9.67 ± 0.58	11.67 ± 2.08
25		Nil	Nil	8.67 ± 1.53	Nil
12.5		Nil	Nil	Nil	Nil
6.25		Nil	Nil	Nil	Nil

Values are presented as mean ± standard deviation of triplicate determination, nil = zero

The evidence of the antibacterial activities of free flavonoids from *P. pterocarpum* stem bark in Table 3 shows that *S. aureus* had highest activity at 100 mg/ml, followed by *B. cereus*, *E. coli* and *S. typhi* in decreasing order. Except *B. Cereus* which had no zone of inhibition at 50 mg/ml, all the test organisms showed zones of inhibition with that of *S. aureus* being the highest. Only *E. coli* showed zone of inhibition at 25 mg/ml while the remaining test organisms showed no zones of inhibitions at 12.5 and 6.25 mg/ml.

DISCUSSION

The phytochemical study of *P. pterocarpum* stem bark revealed its richness in important bioactive compounds such as saponins, alkaloids, terpenoids and flavonoids which are known to possess biological activities [14]. The biological activities of these phytoconstituents have enabled their use in the management of many infectious diseases [15, 16].

In the antibacterial activity studies, the methanol extract and flavonoid extract of *P. pterocarpum* stem bark as well as chloramphenicol (a reference antibiotic drug) showed reasonable but varied antibacterial activities against the pathogenic microorganisms used in this study, as demonstrated by measuring the diameters of inhibition zone. This is consistent with several reports showing the antimicrobial properties of plant phytochemicals under laboratory conditions [17-19].

The high zones of inhibition exhibited by the methanol extract at 100 mg/ml and 50 mg/ml might be due to the effects of numerous important phytochemical constituents present in the methanol extract in which many of the constituents might have acted in synergy to inhibit the test organisms. This supports the earlier reports that plants endowed with phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids and steroids are likely to possess antibacterial properties [20] and are well known for many medicinal and physiological activities [21].

The free flavonoids from *P. pterocarpum* stem was most efficacious among the plant extracts, (exhibiting the widest inhibition zone against both Gram-positive and Gram- negative bacteria) and showed a better antibacterial activity at 25-100 mg/ml against *B. cerus* and *S. typhi*, but had a less activity against *E. coli* and *S. aureus* when compared to the standard drug. The methanol extract had activity against all test organisms except *B. cerus,* but the activity was less than that of chloramphenicol (the standard drug). It is likely that flavonoids, in combination with other phytochemicals, caused the observed effect of the extract. Bound flavonoids showed the least activity against the spectrum of bacteria used, showing activity only against *B. cerus* which was less when compared to the reference standard drug. In the present study, free flavonoids showed a higher antimicrobial activity compared to bound flavonoids. This may be attributed to the functional groups in flavonoids [22, 23]. The selective antibacterial activity observed points to the fact that hydroxyl (OH) and aldehyde (-CHO), among other groups present in flavonoids are very important for their reactions to other molecules as stated by Dzoyem *et al.* [24]. It seemed that binding of other moieties to these functional groups in flavonoids greatly reduced their antibacterial activity [25]. The observed zones of inhibition against test organisms by the methanol and flavonoid extracts showed potential antibacterial activities of these extracts. The results obtained in the present study are in agreement with the findings of previous studies on flavonoids extracted from plants [26, 27]. The variations observed in the susceptibility pattern of the test organisms to the bioactive molecules in this study may be attributed to their chemical nature and to their different mechanisms of antibacterial action. Some flavonoids are known to affect bacterial cell membrane permeability by disrupting the membrane integrity while some others inhibit DNA gyrase, thereby inhibiting DNA synthesis [23].

CONCLUSIONS

The study demonstrated broad spectrum antibacterial activities of the methanol extract, free flavonoids and bound flavonoids of *P. pterocarpum* stem bark against both Gram positive and Gram negative bacteria at high concentrations. The antibacterial activities exhibited by

the plant extracts against the pathogenic test organisms used in this study suggest the potentials of the plant extracts in the therapy of bacterial infections. The findings further revealed that the plant contains phytochemical substances that can be used as components of new antimicrobial agents with potential antibacterial activity. The study has also given credence to the folkloric use of this plant as antibacterial agent.

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