Research Article

In-vitro Antibacterial activity of methanolic extract of *Perquetina nigrescens* (Afzel.) Bullock. leaves and *Thevetia peruviana* (Pers) Schums. roots

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ABSTRACT

Ethnobotanical Investigation has revealed that the roots of *Thevetia peruviana* and leaves of *Parquetina nigrescens* are being used to treat bacterial diseases. Phytochemical screening revealed the presence of alkaloids and terpenoids in both species. Agar well diffusion method was employed in the sensitivity test of the studied ethnobotanicals. Different concentrations (50, 100, 200 and 300mg/ml) of extracts of *T. peruviana* roots and *P. nigrescens* leaf extracts were employed against *E. coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus mutans*. All the organisms showed highest and lowest levels of inhibitions at 300mg/ml and 50mg/ml respectively. Leaf extract of *P. nigrescens* showed relatively more activities than the root extract of *T. peruviana* against the tested organisms. The Minimum Inhibitory concentration of both extracts also showed considerable activity at (50,100 and 200) mg/ml while the growth of the microorganisms was unrestrained at 25mg/ml.

Key words: Bacteriostatic, *Thevetia peruviana, Parquetina nigrescens*, Microorganisms, Phytochemicals

1. INTRODUCTION

Thevetia peruviana, a member of Apocynaceae family commonly known as Yellow oleander, is an ornamental plant growing all around India, China, and Australia. An oil based paint containing the species was reported to protect wood as it exhibits antifungal, antibacterial and anti-termite and anti-inflammatory properties [1, 2]. The molluscidal effect of its latex, stem bark and leaf has also been reported [3]. In addition, its seed cake and oil have been reported for their potentials in agriculture and industrial application [4].

Parquetina nigrescens is a perennial twinner generally grows in the forest and often planted around houses by traditional herbal practitioners in the South-Western part of Nigeria, probably for its

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numerous medicinal applications. Earlier, presence of important phytochemicals such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, phlobatonins and saponins have been reported [5-7]. Also, high level of protein with essential micronutrients was found in this species and has been found to be antisickling in action [7]. It was noted that the plant is used in the treatment of diarrhoea, skin infections and gonorrhea [8]. In addition, its antimicrobial and gastrointestinal protective properties with antioxidant as well as its antidiabetic and haematinic properties have been reported[9-12]. It is also used as a cardiac tonic. It is commonly called *ogbo* among the Yoruba in the South-Western part of Nigeria. Information obtained from traditional medical practitioners in the mentioned region revealed that drinking decoctions of *Parquetina nigrescens* leaf could significantly arrest the progression of tumour proliferation in patients [13]. In view of this, the cytotoxic effects of the species against cancer cells was confirmed [14]. However, toxicity of the latex and leaf sap was reported [15].

Table 1: Profile of studied ethnobotanicals

Sr No	Botanical Name	Family	Common Name	Habit
1	*Parquetina nigrescens*	Asclepiadaceae	Ogbo (Y)	Herb
2	*Thevetia peruviana*	Apocynaceae	Milk Bush (E)	Shrub

Y= Yoruba; E= English

Bacterial infections continue to be the basis significant morbidity and mortality globally, often caused by treatment failure or treatment option restrictions because of the prevalence of antibiotic-resistant isolates [16].Plant based antimicrobials represent a vast untapped source for medicines and, further exploration of plant antimicrobials needs to occur. Antibacterial agents of plant origin have enormous therapeutic potential [17].

With less therapeutic and medical information of the root of *T. peruviana* and *P. Nigrescens* as well as their abundance across the southern part of Nigeria, the present study was designed to reveal their phytochemical contents and antibacterial activity.

2. METHODOLOGY

2.1. Collection and preparation of plant sample

Freshly harvested leaves of *Parquetina nigrescens* and roots of *Thevetia peruviana* were collected from the old Convocation Arena, University of Ilorin. The samples were identified at species level and deposited in the Department of Plant Biology Herbarium, University of Ilorin. The plant samples were thoroughly washed under running water and dried separately at room temperature for three weeks until completely dried. Samples were powdered and stored in air tight containers for further use.

2.2. Extraction of powdered plant sample

Extraction was done using the modified methods of Gbadamosi *et al* [18] and Adeyemi *et al* [19]. 500 gm powder from *P. nigrescens* leaves and *T. peruviana* roots were weighed and macerated in 1000ml of 80% Methanol for 72 hours with intermittent stirring using spatula. The mixtures were filtered using whatman No.1 filter paper and concentrated to dryness using rotary evaporator. The crude extracts were dispensed into labelled sample vials and stored at 4˚C prior further use. Percentage yield of the respective extract was calculated using the formula;

$$\text{Percentage yield} = \frac{\text{Weight of the crude extract (g)}}{\text{Weight of powdered plant (g)}} \times 100$$

2.3. Phytochemical screening

The phytochemical screening of the samples was carried out using standard procedures [18, 20-23].The phytochemicals screened for are alkaloids, flavonoids, saponins, tanins and terpenoids.

2.4. Collection and maintenance of test organisms

The isolates of *Psuedomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, Salmonella typhi* and *Escherichia coli*, used in the present

study were obtained from Microbiology Department, University of Ilorin. The working concentration of bacterial solution was standardized at 10^6 cfu/ml.

2.5. Preparation of culture media and standardization of inoculums

Mueller Hinton Agar, Nutrient Agar and Nutrient Broth were all prepared according to the manufacturer's specifications. Briefly, 38gm of Mueller Hinton Agar was dissolved in 1000 ml of distilled water and then heated until it dissolved completely after which it was autoclaved at 121˚C, 15 lbs pressure for 15 minutes to obtain a reaction of 3.8% w/v aqueous solution at 25˚C and pH of 7.3±0.1.

Nutrient Agar (28gm) was suspended in 1000ml distilled water which was heated to boiling to dissolve the medium completely. It was then sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes to obtain a reaction of 2.8% w/v aqueous solution at 25˚C with a pH of 7.4±0.2.

Nutrient Broth (25 gm) was suspended in 1000 ml purified/distilled waterwhich was heated to boiling to dissolve the medium completely. It was then sterilized by autoclaving at 10lbs pressure (115˚C).

The McFarland nephelometer method was used to standardize the test organisms as described by Albert *et al*[24]. A loop of overnight culture of test organisms was picked into 10ml of sterile Nutrient broth and incubated at 37˚C for 18 hours. After 18 hours, turbidity produced was adjusted to match 0.5% McFarland standards by adding sterile nutrient broth to the overnight culture and matched with 0.5% McFarland standard against a nephelometer (Oxoid, UK). The culture that matches with 0.5% McFarland has bacterial suspension of 1.5×10^8 cfu/ml [24].

2.6. Antibacterial activity analysis

The agar well diffusion method was used to determine the antibacterial activity of roots and leaf extracts of *T. peruviana* and *P. nigrescens* respectively. Sterile Mueller Hinton agar plates were prepared. A sterile cotton swab dipped in 18hrs old standardized culture broth of the test organisms was used to streak the surface of the agar uniformly. A sterile 10mm cork borer was used to bore holes in the inoculated agar and the agar plug was then removed. Using a micropipette, 100 µl each of the concentrations (50, 100, 200 and 300 mg/ml) of roots and leaf extracts of *T. peruviana* and *P. nigrescens* respectively dissolved in 5% DMSO were put into four of the wells. The tests were carried out in three replicates. The plates were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37˚C for 24hours. After incubation, the presence or absence of zones of inhibitions around the wells were observed and recorded, the diameter of the zones of inhibition around each well were measured in millimetre horizontally and vertically along two axes i.e. 90˚ to each other and the mean of the two readings were then calculated [25].

2.7. Determination of minimum inhibitory concentration (MIC)

The method employed in the determination of MIC was broth dilution method [24]. An arithmetic series of dilution was prepared between the concentrations (50, 100, 200 and 300 mg/ml). One millilitre of each concentration was added to each 9 millilitres of sterile nutrient broth containing one millilitre of standardized organism in a test tube. Controls composing of nutrient broth and test organisms in a test tube and only nutrient broth were also prepared to compare turbidity. A total of 8 test tubes were used per extract. The tubes were incubated aerobically in an incubator at 37˚C for 24hrs. The least concentration of the studied extracts that did not permit any visible growth of the inoculated test organism in broth culture was then taken as the minimum inhibitory concentration in each case.

2.8. Statistical analysis

Comparison of means and Analysis of variance (ANOVA) were carried out on all data obtained using Statistical Package for Social Science (SPSS). Differences between means were assessed

for significance at $p<0.05$ by Duncan's Multiple Range Test (DMRT).

3. RESULTS

The percentage yield of the studied ethnobotanicals revealed that *P. nigrescens* had a yield of 13.46% compared to *T. pruviana*(10%)(Table 2).

Table 2: Percentage yield of the plant extracts

	P. nigrescens	*T. peruviana*
Weight of plant material (g)	500	500
Percentage yield (%)	13.46	10.00

The phytochemical analysis of the two plants revealed the presence of Alkaloids, flavonoids and terpenoids. Tannins are present in the roots of *T. peruviana* but absent in *P. nigrescens*. Also, there is presence of saponins in the leaf of *P. nigrescens* but absent in *T. peruviana* (Table 3).

Table 3: Phytochemical screening

Phytochemical Constituents	***P. nigrescens***	***T. peruviana***
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	-
Tannin	-	+
Terpenoid	+	+

+ = Present; - = Absent

The graded concentrations (50, 100, 200 and 300mg/ml) of the leaf extract of *P. nigrescens* and root extract of *T. peruviana* exhibited varying diameters of zone of inhibition around the wells of cultured plates (Table 4).

P. nigrescens at 50mg/ml exhibited lowest antibacterial activity against all the test organisms and highest activity was recorded at 300 mg/ml. The activity exhibited against *E. coli* at 50mg/ml was significantly ($p\leq0.05$) different from the activity exhibited by other graded concentrations. The leaf extract of *P. nigrescens* at 200mg/ml and 300mg/ml had significantly the same activity against *E. coli*. Meanwhile, 100mg/ml had significantly different activity against other graded concentrations.The recorded antibacterial activity of *P nigrescens* against *P. aeruginosa* was also significant especially at 300mg/ml being the highest concentration to exhibit markedly highest inhibition. There is no significant difference between the activity of 100mg/ml and 200mg/ml against the test organisms.

The antibacterial activity exhibited by *P. nigrescens* against *S. mutans* correlates with the one exhibited against *P. aeruginosa* statistically. 300mg/ml exhibited the highest activity of 22.25±1.00 while the lowest activity was observed at 50mg/ml (6.50±1.00). Also, no significant difference between the antibacterial activity exhibited by the studied extracts at 200mg/ml and 100mg/ml likewise between 200mg/ml and 300mg/ml.

The antibacterial activity of *P. nigrescens* against *S. aureus* was also remarkable. *P. nigrescens* leaf extract at 50mg/ml exhibited the lowest inhibition against the test organisms while the highest inhibitory activity was exhibited at 300mg/ml. Though there was no significant difference between the inhibitory activity of 300mg/ml and 200mg/ml but 100mg/ml was significantly different from 50mg/ml as well as 200 and 300mg/ml.

The root of *T. Peruviana* also exhibited antimicrobial activity against the test organisms but not as remarkable as that of *P. nigrescens*. Inhibitory activity against the test organisms increased with increasing concentration of the extract. At 50mg/ml, *T. peruviana* showed little inhibitory activity against all the test organisms, all giving diameters of zone of inhibition of about 1.25mm. For *E. coli*, there were no significant differences in the activity of 50, 100, and 200mg/ml but 300mg/ml showed remarkably significant difference when compared against other graded concentrations.

The activity of *T. peruviana* against *P. aeruginosa* revealed a slightly increased inhibitory activity when compared to that exhibited against *E. coli* at 100mg/ml, 200mg/ml and 300mg/ml. Furthermore, there was no significant difference between the activity exhibited by 100mg/ml and 200mg/ml but 300mg/ml exhibited activity that is significantly different from other graded concentrations.

Table 4: Antibacterial Activity of *P. nigrescens* leaves extract and *T. peruviana* root extract

Diameter of zone of Inhibition (mm) ± S.E.M						
Extract	**Concentration (mg/ml)**	***E. coli* (mm)**	***P. aeruginosa* (mm)**	***S. typhi* (mm)**	***S. aureus* (mm)**	***S. mutans* (mm)**
P. nigrescens Leaves	50	*6.25±1.25^{a}	8.75±0.25^{a}	8.25±0.25^{a}	8.00±0.50^{a}	6.50±1.00^{a}
	100	17.75±0.25^{b}	18.00±0.00^{b}	18.00±0.50^{b}	19.00±0.50^{b}	18.75±0.25^{b}
	200	20.75±2.75^{c}	19.75±0.25^{b}	19.75±1.25^{bc}	22.25±0.25^{c}	21.75±1.25^{b}
	300	21.50±2.50^{b}	22.00±1.00^{c}	22.50±1.00^{c}	22.75±0.25^{c}	22.25±1.00^{c}
T. peruviana Root	50	1.25±0.25^{a}	1.25±0.25^{a}	1.25±0.25^{a}	1.25±0.25^{a}	1.25±0.25^{a}
	100	1.75±0.75^{a}	3.75±1.75^{b}	4.25±1.06^{b}	2.50±0.50^{b}	3.50±0.50^{a}
	200	2.75±0.25^{a}	4.00±2.00^{b}	5.25±1.25^{b}	8.75±0.25^{c}	6.50±2.00^{b}
	300	7.00±3.50^{b}	13.00±2.50^{c}	9.25±0.25^{c}	11.50±0.50^{c}	7.75±1.75^{c}

*Value is Mean±SEM; Values with the same superscript down the column are not significant (p≤0.05)

Table 5: Minimum Inhibitory Concentration (MIC)

	P. nigrescens				***T. peruviana***			
	Broth tubes of MIC (mg/ml)				**Broth tubes of MIC (mg/ml)**			
	25	**50**	**100**	**200**	**25**	**50**	**100**	**200**
E. coli	+	-	-	-	+	-	-	-
P. aeruginosa	+	-	-	-	+	-	-	-
S. typhi	+	-	-	-	+	-	-	-
S. aureus	+	-	-	-	+	-	-	-
S. mutans	+	-	-	-	+	-	-	-

(-): Inhibition (+): No inhibition

The inhibition exhibited by *T. peruviana* against *S. Typhi* at 50mg/ml and 100mg/ml was significantly different. There is no significant difference between the activity exhibited by 100mg/ml and 200mg/ml. 300mg/ml is significantly different from other graded concentrations.

The inhibition exhibited by the *T. peruviana* against *S. aureus* followed the same pattern as one obtained against *P. aeruginosa* and *S. typhi* for 50 and 100mg/ml, though there was no significant difference between 200 and 300mg/ml.

The antimicrobial activity of *T. peruviana* against *S. mutans* revealed that there was no significant difference between 50mg/ml and 100mg/ml. Also, there was significant difference in the activity of 200mg/ml and 300mg/ml against other graded concentrations.

4. DISCUSSION

Phytochemicals are the scientific basis for different activities attributed to the medicinal plant species. Most plants have been reported to conceal diverse of these that are of pharmaceutical importance. The antibacterial activity exhibited by the studied ethnobotanicals may be attributed to the presence of various phytochemicals such as saponins, alkaloids and terpenoids [26, 27]. The presence of flavonoids in the root of *T. peruviana* and leaves of *P. nigrescens* may be responsible for its antimicrobial activity due to their ability to complex with bacterial cell wall [28].

The activity exhibited by *P. nigrescens* corroborates the reports of Odetola, et al., [9]. Also, several researchers have reported that the plant has strong action against gastrointestinal disorder due to its active inhibition against *Salmonella typhi* [9, 29, 30].

The antibacterial activity of *T. peruviana* where similar activity was reported against *E. coli, S. aureus* and *P. aeruginosa* is in agreement with the report of Bhoyar and Biradar[31].There is a reported activity of aqueous extract of *P. nigrescens* against *S. aureus* [9].

Pseudomonas aeruginosa which has been reported to be resistant against various antibiotics [32] was found to be inhibited by the methanolic extract of *P. nigrescens* and *T. peruviana*.

As against the claim of Makanjuola et al., [33] that *E. coli* was not inhibited by the ethanolic extract of *P. nigrescens*, the plant in this study showed remarkable zone of inhibition against *E. coli* which may be due to the different type solvent used for extraction in the respective study [9].

5. CONCLUSION

Leaf of *P. nigrescens* showed higher extract yield than the root of *T. peruviana*. Both extracts showed the presence of alkaloids and flavonoids, but while *P. nigrescens* leaf extract had saponin, it was absent in *T. peruviana. P. nigrescens* show better inhibition against *E. coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Staphylococcus mutans* as compared with *T. peruviana* extracts. This study has demonstrated the antibacterial activity of these plant species, confirming their potency in local medicine and suggesting their prospects in the development of new drugs to fight microorganisms.

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