

Full Length Research Paper

Antimicrobial activity of *Parquetina nigrescens* on some multidrug resistant pathogens isolated from poultry and cases of otitis media in dogs from Nigeria

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There is a high prevalence of multidrug resistant pathogenic bacteria from food and companion animals in Nigeria due to abuse and misuse of antibiotics. The current work was carried out to study the antimicrobial activity of the leave extract of *Parquetina nigrescens* on ten multidrug resistant pathogens including: *Escherichia coli*, *Salmonella enterica* Gallinarum, *Corynebacterium diphtheriae*, *Acinetobacter species*, *Staphylococcus aureus* and *Pseudomonas diminuta* that were resistant to 7 to 10 conventional antibiotics. The ten strains of the organisms tested were either isolated from cases of otitis media in dogs following more than a year of antibiotic misuse and or abuse or from internal organs of poultry that died of septicaemic disease conditions. The tested leaves extract produced activity in only one of the *C. diphtheriae* isolated from ear swab of one of the otitis media cases with 16 ± 0.0 mm zone of inhibition at 100 mg/mL when compared with 30 ± 0.0 mm at 10 μ g/mL of gentamycin (positive control). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested extract for the isolate were 25 and 400 mg/mL respectively as compared to the respective MIC and MBC of 40 and 320 μ g/mL for gentamycin.

Key words: *Parquetina nigrescens*, multidrug-resistant, otitis, poultry, dog, Nigeria.

INTRODUCTION

In recent times, the use of natural plants/products as alternative therapeutic agents for numerous sicknesses and disease conditions has gained acceptance worldwide. For instance, medicinal plants have been shown to contain avalanche of phytochemicals that are beneficial to humans in health and disease (Romano et al., 2012). Such groups of phytochemicals that are in natural products play active roles as healing agents derived from medicinal

plants/vegetables (Fahey et al., 2012). Recently, there has been emergence of resistant microorganisms wide arrays of orthodox drugs. And this has become a source of major concern all over the world (Ibekwe et al., 2000). Hence, the use of medicinal plants/products is becoming increasingly popular. The current study thus evaluated the antimicrobial activities of the leave extracts of *Parquetina nigrescens* as a possible alternative therapeutic agent to

the commonly used antibiotics on ten clinical strains of multidrug resistant bacteria, that were either isolated from septicemic poultry diseases in Oyo, Osun and Ogun States, Nigeria or from cases of otitis media in dogs that did not respond to antibiotics treatments with commonly used antibiotics following over one year of antibiotic misuse/abuse before the dogs were presented to the University of Ibadan Veterinary Teaching Hospital, Oyo State, Nigeria.

P. nigrescens is an herbaceous plant belonging to the family *Asclepiadaceae*. It is usually woody at the base and measures between 7 and 8 m in length. The plant is commonly found growing on ant-hills across the African regions specifically from Senegal to Nigeria, and over the Congo basin down to south tropical Africa (Saba et al., 2010). The beneficial properties and medicinal potentials of *P. nigrescens* have been documented in literature. For instance, it has been shown to ameliorate haemorrhagic anemia (Agbor and Odetola, 2005). The analgesic, anti-inflammatory and antipyretic effects of *P. nigrescens* leaf extract have been documented (Owoyele et al., 2009). The plants have been shown to have haematinic, antidiabetic, cardiotoxic, anti-ulcerative and antioxidant properties (Saba et al., 2010; Datté and Ziegler, 2001; Ozaslan, 2011; Ayoola et al., 2011). There is however, paucity of reports on the antimicrobial effects of *P. nigrescens* as well as its possible effects on multidrug resistant microorganisms, hence, the drive for this study.

MATERIALS AND METHODS

Extract preparation

Freshly harvested leaves of *P. nigrescens* were identified at the Forest Research Institute (FRIN) Ibadan, Nigeria with voucher number FHI 107128. The leaves were air-dried, blended into powder using electric blender/mill grate and then soaked in 300 ml of distilled water for 24 h. The resultant mixture was filtered with cheesecloth and the filtrate was concentrated under reduced pressure at 40°C for 20 min using a rotary evaporator (Gallenkamp UK). The resulting residue, the aqueous extract, was stored at 4°C. One thousand milligram per kilogram (1000 mg/kg) of the extract was prepared by dissolving 20 g of the concentrated extract in 100 ml of distilled water.

Microorganisms

Ten multidrug resistant pathogenic bacteria isolated from veterinary clinical cases that were mostly associated with treatment failures to commonly used antibiotics were used for this study. The following organisms namely; UIVM 1 *Escherichia coli*, UIVM 3 *Corynebacterium diphtheriae*, UIVM4 *E. coli*, UIVM 5 *Pseudomonas diminuta*, and UIVM 10 *Corynebacterium diphtheriae* were isolated from ear swabs processed bacteriologically from cases of otitis media in dogs presented to the Veterinary Teaching Hospital, University of Ibadan following treatment failures after more than one year of antibiotics abuse/misuse, while the bacteria organisms; UIVM 2 *Salmonella Gallinarum*, UIVM 6 *Salmonella Gallinarum*, UIVM 7 *Salmonella Gallinarum*, UIVM 8 *Acinetobacter* species and UIVM9 *Staphylococcus aureus* were isolated from organs like liver, heart and intestines sampled from septicemic poultry disease condi-

tions in Oyo, Osun and Ogun States, Nigeria.

Antibiotic sensitivity test

The *in vitro* antibiotic sensitivity tests were carried out using conventional antimicrobial agents, namely; Septrin; SXT (30µg), Chloramphenicol; CH (30 µg), Sparfloxacin; SP (10 µg), Ciprofloxacin; CPX (10 µg), Amoxicillin: AM (30 µg), Augmentin: AU (30 µg), Gentamycin: CN (10 µg), Pefloxacin: PEF (30 µg), Tarivid: OFX (10 µg), Nitrofurantoin: (300 µg) and Streptomycin: S(30 µg) for Gram negative organisms and Pefloxacin: PEF(30 µg), Gentamycin: CN (10 µg), Ampiclox: APX (30 µg), Zinnacef: Z (20 µg), Amoxicillin: AM (30 µg), Rocephin: R (25 µg), Ciprofloxacin: CPX (10 µg), Streptomycin: S (30 µg), Septrin: SXT (30 µg) and Erythromycin: E (10 µg) for Gram positive organisms as described by Walton (1972) and modified by Adetosoye (1984). Each of the isolates were inoculated into 5 ml sterile nutrient broth and incubated at 37°C for 8 h. A 1:2000 dilution of the culture was made with sterile nutrient broth, while the mixture was shaken vigorously. Subsequently, a diagnostic sensitivity test agar plate was inoculated by flooding with the 1:2000 diluted mixture of the culture. The excess fluid was drained off and the plate was allowed to stand on the bench for 1 h and antibiotic sensitivity discs of the respective antibiotic (Oxoid) were aseptically applied. The test plates were allowed to stand on the bench for 1 h to allow the antimicrobial agents to diffuse into the agar. The plates were then incubated at 37°C for 18 h after which the results were recorded. The zone of inhibitions around each antibiotic disc in each test was compared with the corresponding zone of inhibition around the antibiotic disc for the control organism.

Determination of antimicrobial activities of *P. nigrescens*

Culture methods

Discrete colonies of each of the ten organisms were sub-cultured into sterile Tryptic Soy broth in tubes and were incubated at 37°C overnight. A 1:100 dilution of each culture was subsequently prepared and used for the study.

Screening method for antimicrobial activities of *P. nigrescens*

This was carried out using the agar cup diffusion technique (Adeniyi et al., 2006; Adegoke et al., 2010). One milliliter (1 ml) of 1:100 dilution of an overnight culture of each bacterial isolates was used to seed sterile molten Mueller-Hinton agar medium maintained at 45°C. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to cut equidistant wells on the surface of the agar into which was added 0.1 ml solution of each fraction reconstituted with 50% ethylacetate to final concentrations of 10, 20, 100 and 200 mg/mL, respectively. The plates were incubated at 37°C for 24 h after which diameters of zones of inhibition were measured. 50% ethylacetate was included in each plate as a solvent control, while Gentamycin (10 µg/mL) was used as a positive control. The experiment was performed in triplicates.

Determination of minimum inhibitory concentration (MIC)

The MIC for the bioactive extracts was determined for UIVM 10 that showed activities during the screening test by the agar dilution method (Lajubutu et al., 1995). Different concentrations of the extracts were prepared to final concentration in the range of 200 to 12.5 mg/ml and 40 to 2.5µg/mL for Gentamycin. Two milliliter of the extract from each dilution and from the positive control was mixed



Figure 1. Antimicrobial activity of leaf extract of *P. nigrescens* on multi drug resistant strain of *C. diphtheriae*.

with 18 ml of molten Mueller-Hinton agar and poured into sterile Petri dishes allowing the agar to set. The surface of the agar was allowed to dry before streaking with overnight broth culture of test organism UIVM 10. The plates were incubated at 37°C for 24 h and examined for the presence or absence of growth. The lowest concentration preventing visible growth was taken as the MIC of the extract. All procedures were performed in triplicates.

Determination of minimum bactericidal concentration (MBC)

The MBC for the bioactive extracts was determined by modification of the method of Aibinu et al. (2007). Concentrations higher than and equivalent to the MIC were prepared in Tryptic Soy Broth ranging from 400 to 12.5 mg/mL for the extract and from 320 to 10 µg/mL for the Gentamycin (positive control); 0.5 ml of a 24 h culture of test organism UIVM 10 was added to 4.5 ml of the respective concentrations of the extracts solution as well as the positive control in sterile test tube. The mixture was incubated at 37°C for 24 h after which aliquots of samples were withdrawn. Ten-fold dilutions were made and 0.2 ml of 1:1000 dilutions was transferred onto extract-free sterile Mueller-Hinton agar in Petri dish. The agar plates were incubated at 37°C for 24 h and observed for absence or presence of growth. The minimum concentration preventing visible growth of the organisms was taken as MBC. All procedures were performed in triplicates.

RESULTS

As shown in Table 1, all the ten bacteria used for this study were multidrug resistant to seven of the ten conventional antibiotics. Bacteria isolates UIVM 3, UIVM 9 and UIVM 10 were resistant to the respective seven antibiotics shown, while UIVM 1, UIVM4, UIVM 6, UIVM 7 and UIVM 8 were resistant to the different combinations of eight antibiotics as shown, and the highest level of

resistance to ten of the tested antibiotics was exhibited by UIVM 5.

Also, of all the ten bacteria tested with the extract, only *C. diphtheriae* (UIVM10) isolated from the ear swab of one of the dogs with otitis media had activity for the tested extract with 16±0.0 mm zone of inhibition at 100 mg/mL as compared to the 30±0.0 mm zone of inhibition produced for the same isolate at 10 µg/mL for gentamycin as shown in Figure 1. The respective zone of inhibition produced by 10 µg/mL of gentamycin for UIVM 2, UIVM 5, UIVM6, UIVM 7 and UIVM 9 are shown in Table 1, while UIVM1, UIVM3, UIVM4 and UIVM 8 were resistant. The MIC of the tested extract for UIVM10 was 25 mg/mL and the MBC was 400 mg/mL, while its MIC for gentamycin was 40 µg/mL and MBC was 320 µg/mL.

DISCUSSION

Various beneficial effects of the leaf extracts of *P. nigrescens* such as haematinic, antidiabetic, cardiotoxic, anti-ulcerative and antioxidant properties have been scientifically established and well documented (Saba et al., 2010; Datté and Ziegler, 2001; Ozaslan, 2011; Ayoola et al., 2011). The leave extract has also been reported to produce significant analgesia as evaluated through hot plate and formalin paw licking tests in experimental animal model (Owoyele et al., 2009). It was further established that significant inhibition to various types of inflammation was produced by the same extract (Owoyele et al., 2009). These findings served as a Whereas, the extract did not produce any activity in nine of the multidrug resistant bacteria pathogens used for the screening test as shown in Table 1. There was however a measure of activities in one of the isolate; UIVM10, a *C. diphtheriae* isolate from one of the cases of otitis media with an history of treatment failure with conventional antibiotics following a prolong misuse and or abuse of antibiotics (Figure 1). This observation of antimicrobial activity in the isolate is noteworthy because of the multidrug resistant nature of the isolate to the conventional antibiotics. As shown in Table 1, the organism was originally resistant to seven conventional antibiotics namely: Pefloxacin (30 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxicillin (30 µg), Streptomycin (30 µg) and Septrin (30 µg). There was however a 30±0.0 mm zone of inhibition for Gentamycin at 10 µg/mL when used as positive control using the leaf extracts screening in the agar cup diffusion technique. This observation suggests that the latter technique may be more effective than the disc diffusion method. The multidrug resistant nature of this isolate, UIVM10 was evident in the high value obtained for its MIC and MBC: 25 and 400 mg/mL, respectively. These high values were comparable to the high values for the positive control, gentamycin of 40 µg/mL for the MIC and 320 µg/mL for the MBC. To the best of our knowledge, we are reporting

Table 1. Antimicrobial activities of *P. nigrescens* on some multidrug resistant bacteria pathogens isolated from poultry and unresponsive cases of otitis media in dogs.

Organism code	Organism	Source	Resistant to	Antimicrobial activities of the extract	Activities on 10 µg/ml gentamycin	Gentamycin activity at 10 µg/ml
UIVM 1	<i>Escherichia coli</i>	Dog (otitis media)	Sxt, Sp, Am, Au, Cn, Pef, OfI, Cpx	No activity	Resistant	Resistant
UIVM 2	<i>Salmonella gallinarum</i>	Poultry	Sxt, Ch, Sp, Cpx, Amx Au, Cn, Pef, S	No activity	30±0.0 mm	30±0.0 mm
UIVM 3	<i>Corynebacterium diphtheriae</i>	Dog (otitis media)	Pef, Cn, Apx, Z, Am, S, Sxt	No activity	Resistant	Resistant
UIVM 4	<i>Escherichia coli</i>	Dog (otitis media)	S, SP, Am, Au, Cn, Pef, S, Ch	No activity	Resistant	Resistant
UIVM 5	<i>Pseudomonas diminuta</i>	Dog (otitis media)	Sxt, Ch, Sp, Cpx, Am, Au, Cn, S, Pef, Ofx	No activity	27±0.0 mm	27 ±0.0 mm
UIVM 6	<i>Salmonella gallinarum</i>	Poultry	Sxt, Sp, CPX, Am, Cn, Pef, OfI, St	No activity	25±0.0 mm	25 ±0.0 mm
UIVM 7	<i>Salmonella gallinarum</i>	Poultry	Sxt, Sp, CPx, Am, Cn, Pef, OfI, S	No activity	27±0.0 mm	27 ±0.0 mm
UIVM 8	<i>Acinetobacter species</i>	Poultry	Sxt, SP, Cpx, Am, Cn, Pef, OfI, S	No activity	Resistant	Resistant
UIVM 9	<i>Staphylococcus aureus</i>	Poultry	Sxt, Sp, Cpx, Am, Pef, OfI, S	No activity	25±0.0 mm	25 ±0.0 mm
UIVM 10	<i>Corynebacterium diphtheriae</i>	Dog (otitis media)	Pef, Cn, Apx, Z, Am, S, Sxt	16±0.0 mm at 100 mg/ml	30±0.0 mm	30 ±0.0 mm

Sxt = Septrin, Ch = chloramphenicol, Sp = sparfloxacin, Cpx = ciprofloxacin, Am = amoxicillin, Au = augmentin, Cn = gentamycin, Pef = pefloxacin, Ofx = tarivid, S = streptomycin, Apx = ampiclox, Z = zinnacef, R = rocephin, E = erythromycin, N = nitrofurantoin.

for the first time the antimicrobial activity of the leaf extract of *P. nigrescens*, especially on multidrug resistant pathogenic clinical bacteria isolate. The inability of the extract to produce any activities

on nine of the other isolate might not be unconnected with the highly resistant natures of the strains; as shown in the Table 1, most of them were resistant to more than seven conventional commonly

used antibiotics in Nigeria, including the positive control. This study brings to light, the apparent possible public health risk in terms of treatment failure in animal and possibly in human in Nigeria

due to the characteristics of these pathogens

REFERENCES

- Adegoke OA, Ogunleye AO, Lawal TO, Idowu OS, Adeniyi-Akee MA (2010). Antimicrobial properties of 4-Carboxyl-2, 6-dinitrophenylazohydroxynaphthalenes. *Afr. J. Microbiol. Res.* 4:2444-2450.
- Adeniyi BA, Lawal TO, Olaleye SB (2006). Antimicrobial and gastroprotective activities of *Eucalyptus camaldulensis* crude extracts. *J. Biol. Sci.* 6:1141-1145.
- Adetosoye AI (1984). Studies of *Escherichia coli* with special reference to drug resistance and enterotoxigenicity. A Ph.D thesis in the Department of Veterinary Microbiology and Parasitology, University of Ibadan.
- Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T (2007). Evaluation of the Antimicrobial Property of different parts of *Citrus aurantifolia* (Lime fruit) as used locally. *Afr. J. Trad. Cam.* 4:185-195.
- Agbor GA, Odetola AA (2005). Effect of *Parquetina nigrescens* on erythrocyte indices and serum electrolytes of rats following acute blood loss. *Pak. J. Biol. Sci.* 8:527-531.
- Ayoola AO, Akinloye O, Oguntibeju OO, Oke JM, Odetola AA (2011). Antioxidant activities of *Parquetina nigrescens*. *Afr J Biotechnol.* 10:4920-4925.
- Datté JY, Ziegler A (2001). Pharmacological investigation on nigrescigenin-a cardenolide from *Parquetina nigrescens* (Afzel.) Bullock: comparative studies on cardiotoxic effects of *Parquetina nigrescens*, gastrophanthin and noradrenaline in guinea-pig isolated atria. *J. Pharm. Pharmacol.* 53:859-866.
- Fahey JW, Talalay P, Kensler TW (2012). Notes from the field: "green" chemoprevention as frugal medicine. *Cancer Prev. Res.* 5:179-188.
- Ibekwe VI, Ubochi KC, Anyanwu BN (2000). Prevalence of Penicillin Resistance in organisms that cause sexually transmitted diseases in Port Harcourt, Nigeria. *Int. J. Environ. Health. Res.* 10: 251-255.
- Lajubutu BA, Pinney RJ, Roberts MF, Odelola HA, Oso BA (1995). Antibacterial activity of diosquinone and plumbagin from the Root of *Diospyros mespiliformis* (Hostch) [Ebenaceae]. *Phytother. Res.* 9:346-350.
- Owoyele BV, Nafiu AB, Oyewole IA, Oyewole LA, Soladoye AO (2009). Studies on the analgesic, anti-inflammatory and antipyretic effects of *Parquetina nigrescens* leaf extract. *J. Ethnopharmacol.* 122:86-90.
- Ozaslan M (2011). *Parquetina nigrescens* checks the ulceration and oxidation. *Pak. J. Biol. Sci.* 14:1124-1125.
- Romano M, Vitaglione P, Sellitto S, D'Argenio G (2012). Nutraceuticals for protection and healing of gastrointestinal mucosa. *Curr. Med. Chem.* 19:109-117.
- Saba AB, Oyagbemi AA, Azeez OI (2010). Antidiabetic and haematinic effects of *Parquetina nigrescens* on alloxan induced type-1 diabetes and normocytic normochromic anaemia in Wistar rats. *Afr. Health. Sci.* 10:276-282.
- Walton JR (1972). Bacteriological, biochemical and virulence studies on *Salmonella dublin* from abortion and enteric disease in cattle and sheep. *Vet. Rec.* 90:236-2340.