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Antimicrobial and Gastrointestinal Protective Properties of *Parquetina nigrescens* (Afzel.) Bullock

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Abstract: Leaf extracts of *Parquetina nigrescens* (AAfz.) Bullock, a plant commonly employed for the treatment of gastro-intestinal disorders in Nigeria was tested for antimicrobial activity and gastro-intestinal protective effect. Aqueous leaf extract showed antimicrobial activity against a range of bacteria in the following order of activity: *Staphylococcus aureus* > *Salmonella typhi* > *Proteus mirabilis* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Proteus vulgaris*, whereas the ethanol extract was effective only against *Pseudomonas aeruginosa* and *Salmonella typhi*. The aqueous extract also significantly reduced gastric acid secretion, reduced ethanol-induced gastric ulceration ($p < 0.05$) and caused elevation in gastric mucus secretion. The antimicrobial activity against the common pathogenic microbes *S. aureus*, *S. typhi* and *P. aeruginosa* may account for its acclaimed potency against diarrhea. In addition, its protective effect against oral administration of absolute ethanol reflected by the increased mucus secretion and decreased gastric acid secretion may also be responsible for its claim as having anti-ulcer property.

Key words: *Parquetina nigrescens*, antimicrobial activity, gastro-intestinal protective effect

INTRODUCTION

In Nigeria, as in most developing countries, about 80% of the population still use traditional medicines for the treatment of a wide variety of diseases including gastro-intestinal disorders (GIT) which are very common. Traditionally, diagnoses of the different types of gastro-intestinal diseases are sometimes indistinct as most disorders of the GIT are simply described as stomach ache. Consequently the same medication is often used to treat various types of GIT disorders. *Parquetina nigrescens* (Pn) is one of the herbs commonly used in the South Western part of Nigeria for this purpose. Different parts of the plant (leaves, bark, latex and roots) are also used as constituents of medications used for treatment of diverse diseases such as rickets, diarrhea, skin lesions, menstrual disorders and gonorrhoea (Sofowora, 1993; Adeyemi, 1994). In East Africa, leaf decoction is drunk in the evening as aphrodisiac (Kokwaro, 1993), as an ingredient in herbal preparations for insanity in Nigeria (Iwu, 1993) and for dropsy in India. The plant is also employed for other purposes. In the Congo basin it is used as arrow poison while Cameroonians use the latex as body paint over charcoal.

Phytochemical constituents of the leaf include cardenolites, glycosides and alkaloids (Marks *et al.*, 1975; Burkill, 1997).

Although most of the acclaimed medicinal properties have not been confirmed scientifically, the plant is extensively used in traditional medical practice in the Yoruba speaking part of Nigeria where its Yoruba name is Ewe Ogbo, meaning The leaf that hears. Also, a part of the incantation that precedes its use says ohun ti a ba wi fun ogbo ni ogbo ngbo meaning that whatever we tell ogbo it hears or does (Sofowora, 1993) reflecting the importance and belief in its efficacy for cure of virtually all ailments. Earlier investigations have confirmed its haematinic properties (Agbor and Odetola, 2001; Erah *et al.*, 2003) its cardiogenic and catecholamine-like effects (Dattle and Ziegler, 2001). Its anti-bacterial and protective effect on gastro-intestinal injuries are been reported.

MATERIALS AND METHODS

Animals: The studies were carried out in the Research Laboratories of the Department of Biochemistry, Physiology and Pharmaceutical Microbiology and Clinical Pharmacy, College of Medicine, University of Ibadan, Ibadan, Nigeria between April 2001 and June, 2003.

Adult males albino rats of Wistar strain bred in the animal house of the Physiology Department, University of Ibadan, Nigeria were used. The animals were housed and maintained on standard rats' pellets (Ladokun Feeds Nig. Ltd., Ibadan) with free access to water in the animal house of the Department of Biochemistry. Animals for each test were randomly divided into groups of 6 animals per group.

Drugs and chemicals: Ethanol, Methanol (BDH chemicals), Sucrose, Sodium acetate, Alcian blue, Magnesium chloride (Sigma Chemicals). All other reagents and agar are of analytical grade.

Plant materials: Fresh aerial part of *P. nigrescens* (Pn) was collected on the premises of the University of Ibadan in April, 2001 and authenticated by Prof. A. Egunyomi of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. Voucher specimen was deposited in the departmental herbarium. The leaves were air dried and crushed with laboratory mortar and pestle.

Preparation of plant extract

Aqueous extract: Cold-water infusion was prepared by soaking 250 g of powdered air-dried leaves of *P. nigrescens* in 1 L distilled water for about 24 h with occasional shaking. The infusion was then filtered and the filtrate was distilled under-reduced pressure to a small volume that was then evaporated to a thick brown paste, which was stored at -4°C before use. The percentage yield was 1.62.

Ethanol extract: Two hundred and fifty gram of powdered leaf of Pn was defatted with petroleum ether at temperature between $60-80^{\circ}\text{C}$ and then refluxed with 95% ethanol. Ethanol extract on evaporation in a rotary evaporator yielded a thick dark brown paste. The percentage yield was 2.24.

Microorganisms: All bacteria used were clinical isolates except *Bacillus subtilis* and *Salmonella typhi*.

Antibiotic susceptibility test: This test was carried out in the Department of Pharmaceutical Microbiology using the National Committee for Clinical Laboratory Standard Protocols (1997) to determine antibiogram of all the clinical isolates.

Determination of antimicrobial activity of extracts: The agar diffusion method, previously described by Verpoortee *et al.* (1983) and Adeniyi *et al.* (1995) were used. A 60 μL of each extract was tested in triplicates at two different final concentrations of 6 and 18 mg mL^{-1} ,

respectively. The diameters of the inhibition zones of inhibition (mm) were measured and expressed as means and standard errors of means.

Determination of Minimum Inhibitory Concentrations (MICs): The MICs were measured for biologically active extract using broth micro dilution method. The MICs were read after 24-48 h of incubation at 37°C . The MIC was regarded as the least concentration of the extract that gave no visible growth from a triplicate experiment (Lajubutu *et al.*, 1995).

Ethanol induced gastric ulceration: The method of Rafatullah *et al.* (1995) was used in the examination of effect of *P. nigrescens* on ethanol induced gastric ulceration. Eighteen rats were randomly divided into 3 groups of 6 rats per group. The control group, was fed with standard chow and water given *ad libitum* for 2 weeks. Groups 2 and 3 were fed with standard chow and aqueous extract (100 and 500 mg kg^{-1}), which was given by gavage daily for 2 weeks. After an overnight fast of 12 h, rats in the first group used as the control were each given 1 mL of normal saline by gavage. Rats in the second and third groups were given aqueous extract of *P. nigrescens* at 100 and 500 mg kg^{-1} , respectively also by gavage and left for one hour after which each animal was given 1 mL of absolute alcohol. One hour after alcohol administration, the animals were sacrificed and dissected. The stomachs were brought out and the content flushed out with 10 mL of distilled water into petri dishes for estimation of gastric acid.

Thereafter the stomachs were cut open and examined for gastric lesions by tracing the stomach and ulcerated portion on a glass slide with a marker. The lesion on each rat stomach was expressed as a percentage of the total area of the gastric corpus.

Estimation of total gastric acid secretion: The gastric content was flushed out with distilled water and titrated against 0.001N NaOH using phenolphthalein as the indicator. Titre values were used to calculate the gastric acid concentration.

Gastric wall mucus determination: The method of Shah *et al.* (1997) was used to estimate the gastric wall mucus of the rats after two weeks of pretreatment with Pn extract. The animals were later intoxicated with 60% ethanol. Four groups of 6 rats per group were used. Rats in the first and second groups were each given 1 mL of distilled water while rats in the third and fourth groups were given aqueous extract of *P. nigrescens* at 100 and 500 mg kg^{-1} , respectively for 2 weeks.

Estimation of gastric non-protein sulphhydryl groups (NP-SH):

Two groups of control rats were given distilled water while the other three groups of test rats received extract at 100, 500 and 1000 mg kg⁻¹, respectively for 14 days by oral administration. One hour after the last treatment with extract, all the rats in one of the control groups on distilled water and the three test groups were given 1 mL of 80% ethanol by gastric intubations. One hour after alcohol intoxication, all the animals were killed by stunning. They were dissected and their stomachs removed, rinsed with 1.15% KCl solution, blotted with whatman filter paper, weighed and homogenized in 0.2 M.EDTA buffer.

The homogenate was then analysed for NP-SH using the method of Sedlak and Lindsay (1968).

RESULTS

Antimicrobial activity: This sensitivity pattern of the tested bacterial strains showed 100% resistance to ampicillin, 80% to tetracycline and 70% to co-trimazole. It is important to note that none of the test organisms showed resistance to less than 3 types of antibiotics hence could be term Multiple Drug Resistant (MDR) bacterial. The result of the antibacterial activity (Table 1) revealed that the aqueous extract of *P. nigrescens* was active against a wide range of MDR bacteria though at the highest concentration tested. The methanolic extract (A) was found to have weak activity only on *Pseudomonas aeruginosa* and *Salmonella typhi* but no activity against

other bacteria at the highest concentration tested. The MICs of the aqueous extract (B) ranged between 3 to >18 mg mL⁻¹ while that of the methanol extract (A) was between 15 to >18 mg mL⁻¹.

Ethanol-induced gastric ulceration: Compared with the control animals pre-treated with normal saline, those animals pre-treated with 100 and 500 mg kg⁻¹ of the extract had reduction in the percentage area ulcerated in their stomach. Though those animals that received 100 mg kg⁻¹ of extract showed marked reduction in area ulcerated the reduction failed to attain significance (p>0.05), while those that received 500 mg kg⁻¹ of extract had significantly reduced percentage of area ulcerated (p<0.05) when both were compared with control (Table 2).

Gastric acid secretory study: The acid secretory responses in animals pre-treated with 100 and 50 mg kg⁻¹ body weight aqueous extract of *P. nigrescens* decreased with increasing doses. This decrease became significant at the higher dose of 500 mg kg⁻¹ where the control value in normal rats dropped from 2.53±0.17 to 0.98±0.05 in the test (Table 2).

Gastric mucus secretory study: Aqueous extract of *P. nigrescens* increased the quantity of gastric mucus at the two doses used (Table 3). The increase was more significant at the higher dose (p>0.05).

Gastric wall NP-SH assay: Ethanol (80%) significantly reduced the concentration of NP-SH from 2.10±0.30 in the control to 0.26±0.06, (p<0.05) (Table 4).

Table 1: *In vitro* antimicrobial activity of extracts of *Parquetina nigrescens*

| Microorganism | Antibiogram (Resistant pattern to antibiotics) | Diameter zone of inhibition (mm)* and MIC (mg)* | | | | | | | |
|--------------------------------|--|---|--------|-----|---------------------------------------|--------|------|----------|-------------------------------------|
| | | Methanol extract (Concentration in mg) | | | Aqueous extract (Concentration in mg) | | | Controls | |
| | | 6 | 18 | MIC | 6 | 18 | MIC | 50% Meth | Gentamicin (5 µg mL ⁻¹) |
| <i>Staphylococcus aureus</i> | Pen, Amp., Cro. | R | R | >18 | 11±0.3 | 20±0.3 | 3 | - | 20±0.3 |
| <i>S. albus</i> | Pen., Amp., Tet., Col. | R | R | >18 | R | 12±0.5 | 15 | - | 18±0.2 |
| <i>Bacillus subtilis</i> | NCTC | R | R | >18 | R | 12±0.3 | 15 | - | 20±0.3 |
| <i>Escherichia coli</i> | Amp., Cot., Tet. | R | R | >18 | R | R | >18 | - | 17±0.1 |
| <i>E. coli</i> | Amp., Cot., Col. | R | R | >18 | R | R | >18 | - | 18±0.4 |
| <i>Pseudomonas aeruginosa</i> | Amp., Cot., Tet., Col., Str. | R | R | >18 | R | 12±0.3 | 12 | - | R |
| <i>P. aeruginosa</i> | Aug., Nit., Pef., Cip., Nal. | | | | | | | | |
| | Cro., Cot., Nal., Nit., Tet., Amp. | R | 10±0.4 | 15 | R | 12±0.7 | 10.5 | - | 12±0.3 |
| <i>Proteus mirabilis</i> | Amp., Tet., Co. | R | R | >18 | R | 12±0.2 | 9 | - | 14±0.2 |
| <i>Proteus spp.</i> | Pef., Cip., Gen., Cot. Tet., Amp. | R | R | >18 | R | R | 15 | - | 10±0.2 |
| <i>Klebsiella edwardsiella</i> | Gen., Cot., Amp., Tet. | R | R | >18 | R | R | >18 | - | R |
| <i>Klebsiella sp.</i> | Amp., Cot., Tet. | R | R | >18 | R | 12±0.5 | >18 | - | 10±0.2 |
| <i>Salmonella typhi</i> | NCTC | R | 10±0.2 | 15 | 9±0.2 | 17±0.5 | 6 | - | 12±0.3 |

Note: Pen. = Penicillin, Amp. = Ampicillin, Cot. = Co-trimazole, Tet. = Tetracycline, Co = Colistin, Gen. = Gentamicin, Str. = Streptomycin, Aug. = Augmentin, Pef. = Pefloaxillin, Cip. = Ciprofloxacin, Nal. = Nalidixic acid, Azm. = Azitromycin, Cro. = Ceftriaxole, Meth. = Methanol, MIC. = Minimum Inhibitory Concentration of each extract; *Results are average of triplicate experiment

Table 2: Effects of aqueous extract of *Parquetina nigrescens* (Pn) on ethanol- induced gastric ulceration and gastric acid secretion in rats

| Groups | Treatments | No. of animals | % of area ulcerated | Total gastric acid secretion ($\mu\text{eq}/10 \text{ mins}$) |
|--------|----------------------------|----------------|---------------------|---|
| 1 | Control (NS) | 6 | 26.48±5.41 | 2.53±0.17 |
| 2 | 100 mg kg ⁻¹ Pn | 6 | 12.67±3.78 | 2.15±0.16 |
| 3 | 500 mg kg ⁻¹ Pn | 6 | 6.50±3.12* | 0.98±0.05* |

*p<0.05 significantly different from control, Values in columns 4 and 5 are in Means±SEM

Table 3: Effects of aqueous extract of *Parquetina nigrescens* (Pn) on gastric mucus secretion in rats

| Groups | Treatments | No. of animals | Gastric mucus Secretion (mg g ⁻¹) |
|--------|---------------------------------------|----------------|---|
| 1 | Water | 6 | 0.091±0.008 |
| 2 | 80% Ethanol | 6 | 0.062±0.012 |
| 3 | 100 mg kg ⁻¹ Pn | 6 | 0.093±0.009 |
| 4 | Pn+80% Ethanol | 6 | 0.126±0.008* |
| | 500 g kg ⁻¹ Pn+80% Ethanol | | |

*p<0.05 significantly different from control, Values in column 4 are in Mean±SEM

Table 4: Effect of aqueous extract of *Parquetina nigrescens* (Pn) on non-protein sulphhydryl group (NP-SH) in the gastric wall of rats

| Group | No. of animals | Treatments | NP-SH (mol g ⁻¹ tissue) |
|-------|----------------|--|------------------------------------|
| 1 | 6 | Water | 2.10±0.30 |
| 2 | 6 | 80% Ethanol | 0.26±0.06* |
| 3 | 6 | 80% Ethanol+ 100 mg kg ⁻¹ Pn | 0.45±0.02** |
| 4 | 6 | 80% Ethanol+ 300 mg kg ⁻¹ Pn | 0.41±0.05** |
| 5 | 6 | 80% Ethanol+ 1000 mg kg ⁻¹ Pn | 0.49±0.04** |

n = 6, *p<0.05 significantly different from water treated control, **p<0.05 significantly different from ethanol control

The concentration of NP-SH in animals pretreatment with Pn extract (1000 mg kg⁻¹) was about 88% higher that value in the control group. The increase was however not dose related as there was no significant difference between the doses of extract used.

DISCUSSION

The results of the antibiotic sensitivity pattern is in agreement with recent reports from resistance studies (Goldmann *et al.*, 1996) which indicated that most Gram-positive bacteria species especially staphylococci isolates in the community and virtually all hospital isolates are resistant to penicillins. This pattern suggests that R-plasmid DNA acquisition may be the basis of resistance in the isolates used for antimicrobial screening (Smith and Armour, 1966). It is interesting to note that both extracts especially the aqueous extracts are unusually more effective against the Gram negative than the Gram positive.

The aqueous extract of *Parquetina nigrescens* was active against *Staph. aureus* which has been implicated in many hospital acquired infections. The antibiotic resistance of *Staph. aureus* has become a matter of concern as methicillin resistant *Staph. aureus* (MRSA)

have become very common (Prescott *et al.*, 1999). New drugs of plant origin such as *P. nigrescens* could be a solution to the problem of persistent drug resistance.

Pseudomonas aeruginosa which is known for its resistance to various synthetic antibiotics (Pelczar *et al.*, 1998) was susceptible to both the methanol and aqueous extracts.

The activity of the extracts of *P. nigrescens* against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* may be attributed to the presence of triterpenoids in the plants. Triterpenoids were reported to possess antimicrobial activity against these organisms (Hufford *et al.*, 1993; Batista *et al.*, 1994).

The aqueous extracts of *P. nigrescens* had activity against *Proteus mirabilis* which have been implicated with urinary tract infections and wound infections.

Inhibition of gastric acid secretion and elevation in gastric mucus secretion by Pn aqueous extract is evident from this study (Table 2). The reduction in gastric acid output in animals pre-treated with extract shows that the extract might be acting like the standard drugs ranitidine or cimetidine, which are both anti-secretory agents. The other result, which indicated significant increase in gastric mucus secretion (Table 3) is similar to the effect seen in drugs which increase gastric mucus secretion. One such drug is carbenoxolone sodium, which reduces the turn over rate of the gastric epithelial cell, thereby enabling more cells to reach a mature stage where mucus formation is facilitated. Carbenoxolone also directly stimulates the formation of gastric mucus (Parke, 1976).

Ingestion of alcohol has been shown to increase lipid peroxidation resulting in membrane damage and depletion of gastric mucosal NP-SH (Shah *et al.*, 1997; Al-Harbi *et al.*, 1994). In this study, depletion of gastric NP-SH by ingestion of 80% ethanol was highly significant when compared with values in the normal rats. This depletion was however reduced by about 88% in all groups of rats pre-treated with extract of Pn. Since NP-SH is considered a good measure of GSH (Shah *et al.*, 1997), the higher levels of NP-SH in Pn treated rats is indicative of increased GSH activity. This can be linked, in part, with increased protection of the gastric mucosa against the necrotizing effect of alcohol as shown by significantly lower ulcer index in Pn treated rats. Protective properties of other natural products such as rutin, *Zazyphus sativa* and *Caralluna tuberculata* (Al-Harbi *et al.*, 1994; Shah *et al.*, 1997; La Cassa *et al.*, 2000) have been attributed to their anti-oxidant properties traceable to elevated GSH or NP-SH concentrations in treated animals.

The results of this study clearly show that the extract acts as an anti-ulcerogenic agent by influencing the growth and the development of the gastric mucosa and reducing the basal gastric acid secretion. The extract also exhibited some anti-oxidant properties by preventing depletion of gastric mucosa NP-SH by ethanol to some

extent. These extracts contain substances, which can penetrate the cell wall of Gram-positive bacterial species. These substances may have great potential as therapeutic substances for antibiotic agents.

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