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Research Paper

Antibacterial Potentials of *Parquetina nigrescens* extracts on Some Selected Pathogenic Bacteria

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ABSTRACT

The present study details with the antibacterial assay of therapeutic importance from *Parquetina nigrescens*, a useful medicinal plant. The crude aqueous and ethanol extracts of the plant leaves were assay for antibacterial activity. Some of the test organisms were found resistant to the aqueous extract concentrations while *E. coli* was found resistant to the ethanol extract in all the concentrations Considered. *S. aureus* was the most susceptible to the extracts concentrations considered where 100mg/ml of aqueous inhibited the organism with halo >12mm and >20mm with 300mg/ml of the ethanol extract. Methycilin the reference antibiotic had between 17-26mm inhibitory zones on the tested organisms. The MIC of the aqueous extract concentration was between 25- 50mg/ml while it was between 12.5-50.0mg/ml with the ethanol extract on the inhibited organisms.

Keywords: Crude; Antibacterial; Pathogenic; *Parquetina nigrescens* extracts.

INTRODUCTION

Plants are indeed the first source for preparing remedies in this form of alternative medicine (Ruiz-Teran, et al., 2008).

The search for plants with antibacterial activity has gained increasing importance in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effects of some antibiotics (Soberon, et al., 2007).With the advent of ever-increasing resistant bacterial and yeast strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics (Soberon, et al., 2007) may constitute a reservoir of new antimicrobial substances to be discovered. About 80% of developing countries, citizens used traditional medicine based on plant products.

Parquetina nigrescens is a tree so common in Nigeria and are planted around houses as wind breaker, ornamental and for its value in folk medicine. The plant parts (leaves, bark, latex and roots) are used as constituents of medications for the treatment of diverse diseases (Akinyemi, et al., 2005). Therefore, this study is aimed at the evaluation of the antimicrobial activities of the crude aqueous and ethanol extracts of

Parquetina nigresens and its phytochemicals constituents as a review of its perfect use as antimicrobial agents even as of old.

MATERIALS AND METHODS

Experiment: Random extract concentrations of 100, 250 and 300mg/ml were selected in the study for both aqueous and ethanol extracts. This may help evaluate the appropriate extraction solvent and the concentration(s) for effective therapeutic and pharmaceutical virtue hence the traditional users of this plant administers un-quantified dose of decoctions and concoctions. Such evaluations will suggest the ideal of rightful use of this plant extract in folk medicine for acceptability as over dose will be minimized for any side effect that might result.

***Test Organism*:** The bacterial isolates used in this study are *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus feacalis* and *Escherichia coli* obtained from Microbiology Department, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. Each bacterial solution was standardized at 10^6cfu/ml. Mueller-Hinton agar was used for testing the antibacterial activity.

***Collection and Preparation of Plant Samples*:** The plant leaves were collected from a compound in Akure metropolis where it is serving as an ornamental plant. The leaves were washed and dried in room temperature (27 ± 2^0 C) for 3 weeks. The dried leaves grinded to powder were weighed in two separate containers for ethanol and hot water extract. The extraction was done with the help of apparatus. The extraction was allowed for 72 h at room temperature (27 ± 2^0 C). After extraction, filtration was done with a 3 fold sterile muslin cloth. The filtrates were vaporized to dryness using rotary evaporator (Resona, Germany). The dried extracts were weighed as percentage yield and preserved in sterile bottles at 4 ^{0}C before use.

***Antibacterial Activity*:** The crude aqueous extracts was prepared by reconstituting with sterile distilled water while the ethanol extract was prepared in 5% v/v aqueous dimethyl sulphoxide (DMSO) at concentrations of 100-300mg/ml. The inocula of bacteria organisms were prepared from 24h old broth cultures. The absorbance was read at 530nm and adjusted with sterile distilled water to match that of a 0.5 Mac farland standard solution. From the prepared bacterial solutions, other dilutions with sterile distilled water were prepared to give a final concentration of 10^6 colony forming unit (Cfu) per milliliter. 0.5ml each of the bacterial suspensions was separately plated by spread plating technique. The plates were allowed to stand for 1.5 h for the inoculated bacteria organisms to be established in the medium. Wells of 10.5m dept were made on the seeded plates. The various concentrations of the extracts were introduced into each well using sterile syringe. The plates were labeled and incubated at 37^0 C for 24-48 h. After incubation, clear zones of inhibition around the wells indicates the sensitivity rate of the test bacteria to each extract and diameter of the clear zones were taken as index of the degree of sensitivity by measuring with caliper. Tests were carried out in triplicates. Methicilin at 10µg/ml was used as positive control and 5% equous DMSO as a negative control.

***Minimum Inhibitory Concentration (MIC) Determination*:** 1ml of the inhibited extract solution of the treatments of 100-300mg/ ml were added to 1ml of Mueller Hinton Broth (MHB) and subsequently transferred. 1ml from the first test tube to the next, for up to the seventh test tube. Then 1ml of 24 h old culture of test bacterial organisms (1.0×10^6 cell/ml) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37 ^{0}C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC. The same procedure was repeated using 3.125-50µg/ml. Positive MIC tubes were plated on

freshly prepared nutrient agar and incubated for growth to ascertain the extracts Minimum Bactericidal Concentration (MBC).

***Statistical analysis*:** The inhibitory zones of crude water and ethanol extracts were expressed as the mean $\pm$ standard deviation and compared using student-Waller Ducan test at '*P*' $\leq$ 0.05. For the same treatment, values affected by the same superscripts letter (a-d) are not significantly different (test of student-Waller-Ducan at $P > 0.05$).

Phytocehmical Screening

Qualitative Analysis

***Saponin*:** 2.5gm of the powdered sample was boiled in 25ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

***Flavonoids*:** A portion of the powdered leaves sample was separately heated with 10ml of ethyl acetate in a water bath for 3 min. the mixtures were filtered and 4ml of filtrate was shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids.

***Tannins*:** 0.5gm of powdered sample was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour.

***Phlobatannins*:** Deposition of a red precipitate when on aqueous extract of powdered leaves sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of photatinnius.

***Anthraquinones*:** 0.5gm of the extract was shaken with 100ml of benzene and filtered. 5ml of 10% ammonia was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the lower phase of the ammonia indicates the presence of free anthraquinones.

RESULTS

Positive result was however observed with both the aqueous and ethanol extracts of *Parquetina nigrescens* on the tested organisms. Though, both extracts acted on the test bacterial organisms at different concentrations, with various degree of inhibition, which were varied based on the susceptibility of the organisms to the extracts concentrations (Tables 1A and 1B). Four of the test organisms (*P.aeruginosa, K.pneumoniae, E.,coli* and *E.feacalis*) were not susceptible to 100mg/ml of the aqueous extract. Likewise, these same organisms except *Pseudomonas aeruginosa* proved their resistance to the aqueous extract of the concentrations considered. However, *S.aureus, P.mirabilis* and *S.typhi* were inhibited effectively by both the aqueous and ethanol extracts at all concentrations. The ethanol concentration exhibited more inhibitory potentials on the tested organisms except *E.coli* which could not be inhibited as it proved its resistance ability even to the highest concentration (300mg/ml) of *P.nigresence* employed in this study. However, *E.coli* was much more inhibited by the positive control drug (methycilin) than some of the tested bacterial organisms inhibited by both the aqueous and ethanol extracts (Table 1B).

The MIC and MBC results on the bacterial organisms are summarized in Tables 2; 3. The order of bacterial susceptibility in the decreased concentrations of each extract to 12.5mg/ml was in the order of magnitude in concentrations used in the antibacterial assay. The tested bacterial organisms inhibited by the aqueous extracts concentration demonstrated a low MIC of between 50-25mg/ml (Table 2) and 100-25mg/ml (Table 3) for effective bactericidal action. The ethanol extract concentration MIC though was

low (50mg/ml) in some organisms, but was as high to 12.5mg/ml in most cases that proved their bactericidal action.

DISCUSSION

Differences in inhibitory activities observed in the varied concentrations of the aqueous and ethanol extracts can be linked to the extraction ability of the solvents employed (Tamokou, et al., 2008). Hence the ethanol was able to extract and dissolve the antimicrobial metabolites present in the leaves of *P. nigrescens*, the fully dissolved antimicrobial agents were reproducible in all the concentrations considered, as evident by the zones of inhibition over the aqueous extract (Mellissa, et al., 2005). These observations can be rationalized in terms of the polarity of the extractable bioactive compounds and the ability of the bioactive compounds to diffuse in the culture media used for visible susceptibility reaction by the test organisms to the extracts. Higher inhibitory potentials were exhibited at 300mg/ml concentration.

Staphylococcus aureus the only Gram positive bacteria used among the tested organisms was the most inhibited. In contrary, *E coli* which among other Gram negative tested bacterial isolates could not be inhibited either by the aqueous or ethanol extract. Similar result was reported by Tamokou, et al. 2008; Akrum, et al. 2009. This observation may be due to the formation of slimy protective layer by *E. coli*. Some of the tested bacterial isolates such as *P. aeruginosa* and *K. pneumoniae* are reported to be associated with nosocomial and community acquired infections. The resistance activities demonstrated by these organisms mainly to the aqueous extract concentrations has been shown in some other plant extracts (Adoni, 2006; Tamokou, et al., 2008) of which their inhibitory activities were detrimental to other organisms even as reflected in this study. This proves that these organisms are possessed with resistance factors which might poise a major health concern mainly in the rural and urban areas where modern health cares are in partial, non-operational or not in adoption. It is interesting to know that high aqueous and ethanol extracts concentrations in this study demonstrated on them inhibitory potency to valuable standards that approves any plant extract as being antimicrobial (>2.5mm). The envisaged problem in the use of medicinal plants is the quantity of extract required to enhance effective therapy. The different concentrations of aqueous and ethanol extracts of *P. nigrescens* evaluates high aqueous concentration of above 250mg/ml and ethanol extract of 100mg/ml which resulted at 50 and 25mg/ml respectively as the MBC affinity. Despite the MIC which was based on the lowest concentration at which positive effects were detected in the agar dilution method, the MBC proved some of the positive MIC results as bacteriostatic. Based on the MBC results, it was possible to establish the concentrations at which the plant crude extracts were bactericidal for total clearance in therapeutic evaluation on the tested bacterial organisms.

The higher methycilin antibacterial potency over the aqueous and ethanol crude extracts of *P. nigrescens* could be attributed to fact that conventional antibiotics and other pharmaceutical products are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures expressing purity and high fractionation which certainly will enhance antimicrobial effect than crude extracts. This observation, however, is in agreement with Lenta, et al. 2007, who reported that crude extracts are liable to contamination and deterioration which reduces them in susceptibility. Tamokuo, et al. (2008) also reported that fractions of antibacterial extracts inhibit better than crude antibacterial extracts.

A comparative result was possible at the three different concentrations using the agar diffusion and agar dilution techniques where it was observed that in all the concentrations, ethanol extracts exhibited $\pm$50 % of inhibition over the aqueous

extracts. It is noted mostly that apart from the less concentrations agar dilution and diffusion suffers, they allow the determination of accurate inhibitory affinity and MIC for several microorganisms at the sane time.

The qualitative study of phytochemical constituents of *P. nigrescens* contained the groups of compounds known to have antibacterial activities (Prescott, et al., 2008; Lino and Deogracious, 2006) and they may act by complexing with extra cellular and soluble proteins as well as cell microorganisms (Mellissa, et al., 2005). The random selection of the extracts doses were based on the fact finding if a lesser dose will work the same like a higher dose in both aqueous and ethanol extracts. A particular dose of plant extracts are not considered for therapeutic applications (Adoni, 2006) to avoid any side effect after a brilliant pharmacological activity of any plant extract, dose selection is necessary for safety application.

CONCLUSION

When compared to the reference antibiotic, this plant aqueous extract of above 250mg/ml and 100mg/ml of ethanol extract proved quite promising for the therapeutic and pharmacological use of *P. nigrescens*. Variations in inhibitory potency of aqueous and ethanol extracts of *P. nigrescens* is as a result of the inhibitory compounds which was more extracted by ethanol from the plant leaves. The study demonstrated that folk medicine can be an alternative to modern medicine to combat pathogens.

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Table-1A: Inhibitory zones (mm) of *P. nigrescens* at different concentrations.

Bacterial isolates	Crude water extract (mg/ml)		
	100	250	300
P. aeruginosa	O^{d}	$6.67+0.58^{d}$	$9.67+1.52^{c}$
K. pneumoniae	O^{c}	O^{c}	O^{c}
E. coli	O^{b}	O^{b}	O^{b}
E. feacalis	O^{d}	O^{d}	O^{d}
S. aureus	$12.18+0.56^{d}$	$13.82+0.56^{dc}$	$15.57+0.56^{c}$
P. mirabilis	$12.30+0.57^{dc}$	$13.62+0.57^{d}$	$13.62+1.52^{d}$
S. typhi	$6.67+0.56^{c}$	$6.67+1.56^{c}$	$8.67+1.56^{dc}$

- The results are the mean values of triplicate tests measured in two directions after 24; 48h incubation at 37^{0}C. Methiciline was used as positive reference drug.

Table- 1B: Inhibitory zones (mm) of *P. nigrescens* at different concentrations.

Crude ethanol extract (mg/ml)			Reference
100	250	300	
$13.33+0.53^{bc}$	$13.57+0.56^{c}$	$18.33+0.56^{b}$	$23.23+1.00^{a}$
$6.33+0.57^{d}$	$8.45+0.57^{c}$	$10.67+1.52^{b}$	$20.00+1.00^{a}$
O^{b}	O^{b}	O^{b}	$22.33+1.00^{b}$
$4.67+0.00^{d}$	$6.00+0.56^{c}$	$9.32+0.56^{b}$	$19.33+1.52^{a}$
$14.81+0.56^{cd}$	$19.32+0.57^{bc}$	$20.67+0.56^{b}$	$26.45+1.52^{a}$
$11.52+1.53^{dc}$	$15.32+0.56^{c}$	$19.65+0.00^{b}$	$17.54+0.66^{a}$
$10.67+1.56^{d}$	$12.22+0.56^{c}$	$15.67+1.56^{b}$	$20.16+1.00^{a}$

- Same as in table-1A.

Table -2: Minimum Inhibitory Concentration (MIC) of aqueous and ethanol extracts of *Perquetina nigrescens.*

Bacterial isolates	100mg/ml	250mg/ml	300mg/ml	Reference
P. aeruginosa	-/12.5	25/12.5	12.5/12.5	0.050/0.050
K. pneumoniae	-/50	-/25	-/25	0.025/0.025
E. feacalis	-/12.5	50.12.5	50/12.5	0.0125/0.0125
S. aureus	50/50	25/12.5	25/12.5	0.050/0.050
S. typhi	50/50	50/12.5	50/12.5	0.050/0.050
E. coli	-	-	-	0.050/0.050
P. mirabilis	50/50	25/25	25/12.5	0.1/0.10

Table- 3: Minimum Bactericidal Concentration (MBC) of aqueous and ethanol extracts of *Perquetina nigrescens.*

Bacterial isolates	100mg/ml	250mg/ml	300mg/ml	Reference
P. aeruginosa	-/2.5	100/25	2.5/12.5	0.050/0.050
K. pneumoniae	-/50	-/25	-/25	0.025/0.025
E. feacalis	-/50	50/12.5	50/12.5	0.0125/0.0125
S. aureus	50/50	25/12.5	25/12.5	0.050/0.050
S. typhi	100/50	50/12.5	50/12.5	0.050/0.050
E. coli	-	-	-	0.050/0.050
P. mirabilis	100/100	50/25	25/12.5	0.10/0.10