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Quantitative Phytochemical and *In-Vitro* Antimicrobial Activity of Aqueous Leaves Extract of Blue Pussy leaf (*Nelsonia Canescens*) (Lam.) Spreng

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ABSTRACT

Currently, there is a renewed interest in drugs of natural origin simply because they are green medicine and green medicine offer safe, effective treatment, with minimal or no side effect, easily availability, cheap and are in great demand in the developed World health care. This study is aimed at evaluating the phytochemical constituents and antimicrobial activity of aqueous leaves extract of blue pussy leaf *Nelsonia canescens*. Phytochemical analysis, cork borer methods, and Tube dilution methods for MIC and MBC were employed to determine the phytochemical constituents and antimicrobial activity of the aqueous leaves extracts of *Nelsonia canescens*. Results obtained reveals the presence of alkaloid, total phenol, flavonoids, saponins and tannins at concentration of 330.00±0.00, 144.60±1.76, 107.04±0.62, 72.50±00 and 42.17±1.17 mg/g) respectively. The antibacterial activity of the plant crude extract active on Escherichia coli had the highest mean zone of inhibition of (20.05±0.05) at 20 mg/ml with the MIC at 1.25 mg/ml and MBC of 10 mg/ml. *Staphylococcus aurus* had a mean zone of inhibition of (18.00±0.00) at 20 mg/ml, with the MIC at 1.25 mg/ml and MBC of 5.0 mg/ml. The plant crude extract was active against Gram-negative bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aurus*, Escherichia coli, and Salmonella, typhi. Gram-positive bacteria, *Staphylococcus aurus* and *Streptococcus pyogenes,* had wide zones of inhibition when compared to the activity of the control drug. The presence of some of the phytochemicals and antibacterial activity could explain their use traditionally for the treatment of a wide range of illnesses.

INTRODUCTION

Plants are natural reservoir of medicinal agents that are almost free of side effects normally caused by synthetic chemicals (Fennell *et al.,* 2004), Medicinal plants play an important role in the health of people living in rural and urban societies (Focho *et al.,* 2009). A number of compounds have been isolated from natural sources and many of these compounds were based on the uses of the agents in traditional medicine (Rizvi *et al.,* 2009). The overuse of synthetic drugs with impurities resulting in a higher incidence of adverse drug reactions has motivated mankind to go back to nature for safe remedies (WHO 1999). The World Health Organization (WHO) estimates that 75 - 80% of the population of developing countries currently use medicinal plants as remedies because of better cultural acceptability, better compatibility with the human body (Kamboj 2000; Yandav and Dixit*,* 2008). A single plant may be used for the treatment of various disease conditions depending on the community. Several ailments including fever, asthma, constipation, esophageal cancer, and hypertension have been treated with traditional medicinal plants (Cousins and Huffman, 2002; Saganuwan, 2010). In Africa, the use of the medicinal plant has been the unique health care for 4000 years, long before the advent of western medicine (Silverthorn *et al.,* 2010). Currently, there is a renewed interest in drugs of natural origin simple, because they are green medicine and green medicine offer safe, effective treatment, with minimal or no side effect, easily available, cheap cost and are in great demand in the developed World health care.

Chemotherapy with effective antibiotic drugs remains the main method to control bacterial infections in the absence of a suitable vaccine treatment (WHO 2011). Unfortunately, the bacterial infections have developed resistance against many of the antibacterial drugs couple with the fact that the newly produced effective anti-bacteria drugs are expensive, especially for most poor Nigerians to afford. These have resulted in therapy failure, hence, the need to continue to explore indigenous knowledge of traditional medicine and therapeutic potential of plants through pharmacological research, bioprospecting and drug discovery.

Nelsonia canescens have been used for a long time in diverse contexts, i.e. as an ornamental plant, antioxidant (Sawadogo *et al.,* 2006) antibacterial, anti-inflammatory, analgesic, purgative, and antispasmodic (Focho *et al.,* 2009). *Nelsonia canescens* (Lam.) Spreng (Family *Acanthaceae*) commonly called blue pussy leaf with the synonyms *Justicia brunelloides* (Lam) (Mahias *et al.,* 2007), is found growing in secondary wet evergreen forests, savannah forests and open disturbed habitats, especially in moist areas along roadsides, trails, and as a weed in agricultural land (McDade, 2012). The genus *Nelsonia* is usually treated in the subfamily Nelsonioideae within the *Acanthaceae* (McDade*,* 2008)**.**

This study is aimed at evaluating the quantitative phytochemical constituents and antimicrobial activity of aqueous leaves extract of blue pussy leaf *Nelsonia canescens*.

MATERIALS AND METHODS

Plant Collection and identification.

The leaf of *Nelsonia canescens* was collected in January 2017 from Banana plantations of the Doko community in Lavun Local Government Area of Niger State, Nigeria. The community is located at a (Latitude (8^0 N and Longitude 5^0 E (Adekun,1978).The leaves were identified and authenticated in the Department of Biology Science, Kebbi State University of Science and Technology Aliero and assigned a voucher number: (V.N.148 A).

Bacterial Strains

The bacteria use for the biological test include the gram-positive Staphylococcus aureus and Streptococcus pyogenes, gram-negative are *Pseudomonas aeruginosa, E. Coli* and *Salmonella typhi*

Plant processing and Extraction.

Fresh leaves of *Nelsonia canescens* were shade-dried for 21 days at room temperature (27 – 29.5°C). The dried leaves were pounded using mortar and pestle into powdered form. One hundred and fifty grams of the plant powder was macerated with 3 L of distilled water with continued shaking agitation at room temperature. The extract was filtered every 24 hours using muslin cloth which was followed by a further filtration using Whatman filter paper No. 1 having a pore size of 0.7 um. The filtrate was further concentrated using the rotary evaporator at 45^0C to give a dark semi-solid extract. The extracts obtained were stored in an air-tight amber bottle and refrigerated at 4^0C prior to use (Mann, 2007).

Quantitative Phytochemical Estimation of the Aqueous leaves extract of *Nelsonia canescens*
Determination of Total Phenol

Zero-point five (0.5) mL (1mg/mL) was oxidized with 2.5 mL of 10% Folin Ciocalteau's reagent (v/v) and neutralized by 2mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was taken at 765nm using the double beam Shimadzu UV spectrophotometer, UV-1800. The total phenolic content was subsequently calculated using Gallic acid as standard **(**Singleton *et al.,* 1999)

Determination of Total Flavonoid

Total flavonoid was determined using aluminum chloride colorimetric method (Chang *et al.,* 2002). Quercetin was used to establish the calibration curve. Exactly 0.5mL of the diluted sample was added into a

test tube containing 1.5ml of methanol, 0.1ml of 10% $AlCl_3$ solution and 0.1ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415nm with a double beam Shimadzu UV spectrophotometer, UV-1800. The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank.

Determination of Total Alkaloids

Zero-point five (0.5g) of the sample was dissolved in 96% ethanol - 20% H_2SO_4 (1:1). 1ml of the filtrate was added to 5mL of 60% tetraoxosulphate (VI) and allowed to stand for 5min. 5mL of 0.5% formaldehyde was added and allowed to stand for 3h. The reading was taken at the absorbance of 565nm. The extinction coefficient (E296, ethanol {ETOH} = $15136M^{-1}cm^{-1}$) of vincristine was used as reference alkaloid (Oloyede, 2005)

Determination of Saponins

Zero-point five (0.5g) of the sample was added to 20ml of 1NHCl and was boiled for 4h. After cooling it was filtered and 50ml of petroleum ether was added to the filtrate for the ether layer and evaporated to dryness. 5ml of acetone ethanol was added to the residue and 0.4mls of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent was added into them followed by 2ml of concentrated H_2SO_4. It was thoroughly mixed after 10min and the absorbance was taken at 490nm. Standard saponin was used to establish the calibration curve (Oloyed, 2005).

Determination of Tannin

Zero-point two (0.2g) of the sample was measured into a 50ml beaker. 20mL of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80^{O}C for 1hr., it was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double-layered Whatman No.41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5ml Folin-Denis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water, mixed well and allowed to stand for 20min for the development of a bluish-green colouration. The absorbances of the tannic acid standard solutions, as well as samples, were read after colour development on a UV-spectrophotometer model UV-1800, at a wavelength of 760nm (Emmanuel *et al.,* 2014).

Antimicrobial activity of *Nelsonia canescens*

The antimicrobial activity of *Nelsonia canescens* was carried out by the cork borer method in which a 6mm sterile cork borer which was sterilized by flame and used to bore four wells in the solidified nutrient agar plates aseptically (Oyeleke *et al.,* 2008). The nutrient agar plates were then inoculated with 3 hours incubated suspension of the bacteria isolates using a sterile swab stick which was seeded evenly on the surface of the agar plate. Each well was filled with the crude extract of *Nelsonia canescens* samples of different concentrations mg/ml i.e. 40mg/ml, 30mg/ml, 20mg/ml and 100mg/ml Ampiclox control and these were repeated for each test organism. The plates were then incubated at 37^{0}C for 24 hours. The inhibition zone around each well was measured.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The tube dilution method was used to determine the MIC and MBC of the active extract. A two-fold and seven series (40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/ml) dilutions of each extract were prepared in Nutrient broth. Zero-point one millilitre (0.1 ml) of each of the standardized test organisms (0.5 McFarland turbidity standard) was added to each dilution. One control tube was also prepared which contain only sterile medium without the test organisms or the extract after which all tubes were incubated in a water bath with a shaker at 37°C for 24 hours. The culture was incubated at 37°C for 24 hours. The lowest concentration of the subcultured medium without visible growth was recorded as the minimum bactericidal concentration (Cheesebrough, 2006).

Statistical analysis

Results were expressed as mean **±** Standard Error of Mean (SEM). All the data were analyzed by one-way ANOVA and differences between the means were assessed with the Ducan Multiple comparison test. Differences were considered significant at $p< 0.05$. All the analyses were carried out using Statistical Package for Social Science (SPSS) version 20 (USA).

RESULTS AND DISCUSSION

Table 1. Quantitative phytochemical analysis of aqueous leaf extract of *N. canescens*

Phytochemicals	Amount (mg/100g)
Alkaloids	107.04±0.62
Total phenols	144.60±1.76
Flavonoids	72.50±4.17
Saponins	330.00±0.00
Tannins	42.17±1.17

Table 2. Mean zones of inhibition of aqueous leaf extract *N. canescens*

Bacteria	20 mg/ml	30 mg/ml	40mg/ml	*Ampiclox(1mg/ml)
S. aureus	18.00±0.00^{a}	19.50±0.50^{a}	19.50±0.50^{a}	19.50±2.50^{a}
P.aeruginosa	18.00±1.00^{b}	19.00±0.00^{ab}	20.50±0.50^{c}	0.00±0.00^{a}
E. coli	20.50±0.50^{b}	19.50±0.50^{b}	24.50±0.50^{c}	16.00±1.00^{a}
S. pyogenes	17.50±0.50^{a}	17.00±1.00^{a}	19.00±0.00^{a}	28.00±1.00^{b}
S. typhi	14.50±0.50^{a}	18.00±1.00^{b}	20.50±0.50^{b}	13.00±1.00^{a}

Values are expressed in mean ± standard error of mean.
Values with the same superscript on the same row have no significant difference at p<0.05
Specification for Ampiclox: ≤ 12 mm (resistant), 13-17 mm (intermediate), ≥ 18 mm (susceptible) (CLSI, 2012).

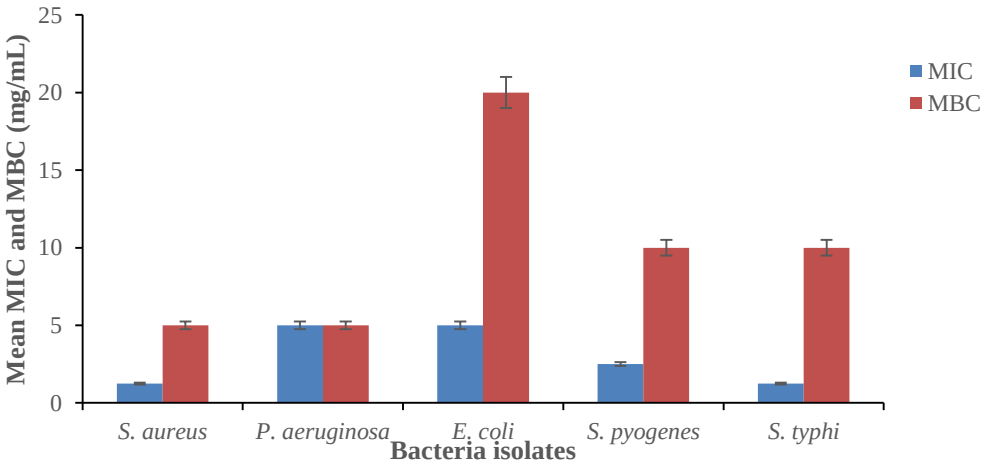

Figure 1. MIC and MBC of aqueous leaf extract of *N. canescens*

DISCUSSION

The results in Table 1 indicated high amount of alkaloids (107.04±0.62mg/g), total Phenol (144.60±1.76 mg/g), flavonoids 72.50±4.17mg/g, saponins (330.00±0.00mg/g) and tannins (42.17±1.17mg/g). These quantitative phytochemicals in aqueous leaves extract of *Nelsonia canescens* in (Table 1) is responsible for preventing infectious diseases and therefore could explain their use traditionally for the treatment of wide ranges of illness including treatment of pain, chickenpox, measles, Inflammations, constipation and gastric ulcer (Owoyele*, et al.,* 2005; Acharya *et al.,* 2012). In Africa, *Nelsonia canescens* is used to reduce fever and as an analgesic in a wide range of conditions including colds, flu, and viral infections (PROTA, 2014). The presence of a variety of phytochemicals in the present study gives the indication that the plant's extracts could be used for curative activity against pathogens and therefore could explain their use traditionally for the treatment of a wide array of illness including malaria (Anaduaka *et al.,* 2013). The presence of alkaloids in plant extracts is also used for a wide range of pharmacological activities including antimalarial, antiasthma, anticancer (Kittakoop *et al.,* 2014).

Results from table 2 and figure 1, reveals the antibacterial activity of the aqueous leaf extract of *Nelsonia canescens* which indicate how active the plant extract is to the test organisms, the result when compared with the standard drug showed statistical significance (P < 0.05), even though the plant extract is still in the crude form. The specification for susceptibility for the standard drug is the zones of inhibition from 18 mm above, the plant extract was observed also to be concentration-dependent such that, increase in the concentration gives a direct increase in the inhibition zones which cut across all the test organisms. *Escherichia coli* was the most susceptible organism having the highest mean zone of inhibition of 20.50±0.50 at 20 mg/ml with the MIC at 5.0 mg/ml and MBC of 20 mg/ml, followed by the *Staphylococcus aurus* with mean zone of inhibition of 18.00±0.00 at 20 mg/ml, this was also evident in the MIC at 1.25 mg/ml and MBC of 5.0 mg/ml. The plant crude extract was active against Gram-negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli,* and *Salmonella, typhi.* Gram-positive bacteria, *Staphylococcus aurus* and *Streptococcus pyogenes* had wide zones of inhibition. This work is in agreement with the finding of (Wayne, 2002). The minimum inhibitory concentrations (MIC) of the crude extract against the test organisms generally were low

suggesting that the plant extract will be highly effective against the test organisms if developed as drugs for the treatment of infections. Therefore it could be concluded that aqueous leaf extract of *Nelsonia canescens* exert its antibacterial (sensitive bacteria) activities against the selected microorganisms and can, therefore, be used to develop drugs that can be employed for the treatment of infections caused by these organisms. Also, the activity of the plant extract is dose-dependent as an increase in activity was observed when the concentration was increased. Since secondary metabolites are usually known to be more active against gram-positive bacterium and mostly inactive against gram-negative bacterium (Agbafor *et al.,* 2011).

CONCLUSION

The present study has shown that the aqueous leaf extract of *Nelsonia canescens* possesses phytochemical constituents. This antibacterial potential is probably due partly to the high contents of alkaloids, total phenolics, flavonoids, saponins, and tannins. These scientific data allows us to justify the traditional use of *Nelsonia canescens* for treatment of pain, reduce fever, inflammation, constipation and gastric ulcer. Further studies will involve the identification of the active ingredients and isolations of the functional groups present in the plant extract.

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