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Median Lethal Dose and Phytochemical Studies 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 offers safe, effective treatment, minimal or no side effects, easily available, lesser cost and are in great demand in the developed world health care. There is decreasing in the efficacy of many modern drugs used for the control of many infections, also an increase in resistance by several bacteria to various antibiotics and increasing cost of prescribed drugs. This study aims at investigating the phytochemical constituents, and toxicity studies of the aqueous leaves extract of *Nelsonia canescens*. Results of qualitative phytochemical screening of the aqueous leaves extract of *N. canescens* showed the presence of alkaloids, phenols, tannins, flavonoids and saponins. The acute toxicity test of the extract *N. canescens* revealed an oral LD50>2000 mg/kg body weight in mice. The presence of some of the phytochemicals, and the values of the LD50 could explains the plant is being used traditionally for the treatment of a wide range of illnesses such as fever, pain, chickenpox, measles, constipation and gastric ulcer without reports of unwanted effects.

Keywords: Acute; Aqueous; Extracts; Medicine; Mice; Phytochemicals; Toxicity

Introduction

Plants are natural reservoir of medicinal agents almost free from side effects normally caused by synthetic chemicals, Medicinal plants play an important role in the health of people living in rural societies. A number of modern drugs have been isolated from natural sources and many of these isolations were based on the uses of the agents in traditional medicine [1-3]. The over use of synthetic drugs with impurities resulting in higher incidences of adverse drug reactions, has motivated mankind to go back to nature for safe remedies [4]. The World Health Organization (WHO) estimates that over 80% of the populations of developing countries currently use medicinal plants as remedies because of better cultural acceptability, better compatibility with human body, etc., [5,6]. A number of diseases including fever, asthma, constipation, esophageal cancer and hypertension have been treated successfully with herbs [7,8]. In African the use of medicinal plant has been the unique health care for 4,000 years, long before the advent of western medicine [9]. Currently, there is a renewed interest in drugs of natural origin simply because they are green medicine, and green medicine offer safe, effective treatment, minimal or no side effect, easily available, lesser cost and are in great demand in the developed world health care.

Nelsonia canescens (Lam) Spreng. (Family *Acanthaceae*) commonly called blue pussy leaf with the synonyms Justicia brunelloides (Lam) [10], is found growing in secondary wet evergreen forests, savannah forests in open disturbed habitats, especially in moist areas along road sides, trails, and as a weed in agricultural land [11]. The genus *Nelsonia* is classified in the sub-family *Nelsonioideae* within the *Acanthaceae*, and has shown to be monophyletic and to comprise the basal lineage among clades of *Acanthaceae* [12].

Materials and Methods

Sample collection

The leaves of *N. canescens* used in this work were harvested in January, 2017 from Banana plantations of Doko community in Lavun, Local Government Area of Niger state, Nigeria. The plant was identified and name authenticated in the Department of the Biological Science, Kebbi State University of Science and Technology Aliero by a Plant Taxonomist Dr. D. Singh. A voucher specimen was deposited at the herbarium and was given a voucher number 148 A.

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Table 1: Qualitative Phytochemical Screening of the Aqueous Leafs Extract of *Nelsonia canescens*.

Phytochemicals	Observation
Total Phenol	++
Alkaloids	++
Tannins	+
Flavonoids	+
Saponins	+++
Steroids	-
Glycosides	-
Phlobatannins	-

+: Present; -: Absent

Animal husbandry: Thirteen healthy adult Swiss albino mice of both sexes were used for this study. They were obtained from Niger State Polytechnic Zungeru, and were acclimatized for one (1) week in the Biological Garden of School of Science, Federal University of Science and Technology, Minna Niger state. The mice were housed in ventilated plastic cages, fed with pellet, deprived of feed overnight before the administration of test substance and had access to potable water throughout the period of the study.

Extract preparation

The fresh leaves of *Nelsonia canescens* were shade-dried for 21 days at room temperature (27°C to 29.5°C). The dried leaves were pounded using pestle and mortar into powdered form at the Centre for Genetic Engineering and Biotechnology (Drug and Vaccine Discovery Unit), Federal University of Technology Minna, Niger State, Nigeria. A 150 g of the plant powder was soaked in 3 L of distilled water for 72 hours and filtered using muslin cloth which was followed by a further filtration using whatman filter paper No.1 with pore size of 0.7 µm. The solvent was removed at 450°C using a rotary evaporator to give a dark solid extract which weighed 23.7 g. The extract obtained was stored in an air-tight amber bottle and kept under refrigeration at 40°C prior to further analysis [13].

Phytochemical screening

Phytochemical screening of the extract of *N. Canescens* was conducted based on coloration and precipitation tests using standard methods of Edeoga et al. (2005); Sofowora [14]; Trease and Evans [15,16] and Harborne [17] (Table 1).

Test for flavonoids: A 2 ml of 10% sodium hydroxide was added to 2 ml of the extract in a test tube. A yellow color which turned color less upon addition of 2 ml of dilute hydrochloric acid was an indication a positive result [16].

Test for phenols: A 2 ml of the extract was mixed with few drops of 10% ferric solution. A greenish blue or violet or blue coloration was an indication of a positive result [16].

Test for tannins: About 5 drops of 0.1% of ferric chloride ($FeCl_3$) was added to 2 ml of the extract. A brownish green or blue black coloration was an indication of a positive result [14].

Test for saponin: A 2 ml of the extract was diluted with 2 ml distilled water. The mixture was agitated in a test tube for 4 min. Appearance of about 1 mm layer of foam indicated a positive result [18].

Test for phlobatannins: A 2 ml of the extract was boiled with 1% aqueous hydrochloride. Deposition of a red precipitate indicated a positive result [15].

Test for alkaloids: A 2 ml of the extract +2 ml of 10% HCL, to the acidic medium, 2 ml of Meyer's reagent was added. Formation of an orange precipitate indicated a positive result [18].

Test for terpenoids: A 2 ml of the extract was mixed with 2 ml of chloroform and 1 ml of concentrated sulphuric acid was carefully added to form a layer. A clear upper and lower with a reddish green inter-phase indicated a positive result [18].

Test for steroids: A 2 ml of the extract was dissolved in 10 ml of chloroform and then 1 ml of concentrated sulphuric acid was added by the side of the test tube. Formation of a reddish upper layer and yellow sulphuric acid layer with green fluorescence indicated a positive result [18].

Test for anthraquinones: A 2 ml of the extract was boiled with 5 ml of 10% HCL for 3 min 5 ml of chloroform was then added followed by further addition of 5 drops of 10% ammonia. A rose pink coloration indicates a positive result [19].

Test for glycosides

A 2 ml of acetic acid, 2 ml of the extract was added. The mixture was cooled in a cold water bath, and then 2 ml of concentrated sulphuric acid was added. Color development from blue to bluish green indicated a positive result [14].

Acute toxicity test

Acute toxicity test of the plant extract was carried out using the method of Lorke [19] as described in Latha and Reddy (2009). In the first phase of the experiment, the animals were randomly divided into three groups of three mice each and were given the plant extract of 10, 100 and 1,000 mg/kg body weight respectively *via* oral route. The mice were placed under close observation for 24 hours to monitor all vital signs, behaviors and any mortality before the commencement of the second phase. In phase two, the animals were grouped into three of one animal each and were orally given higher doses of the plant extract of 1500, 1750 and 2,000 mg/kg body weight. The animals were also observed separately for 24 hours for vital signs, toxicity and mortality.

Then the LD50 is calculated by the formula:

$LD_{50}=\sqrt{(D_0 \times D_{100})}$

D_0=High dose that gave no mortality,

D_{100}=Lower dose that gave no mortality.

Results and Discussion

The results of this study demonstrates that phytochemical screening of *Nelsonia canescens* aqueous leaves extract contains alkaloids, phenols, tannins, flavonoids and saponins in various quantities. Alkaloid is a plant derived compound that is reported to be physiologically active and toxic as well, contains Nitrogen in a heterocyclic ring with complex structure. Alkaloids are reported to be formed as metabolic by-products and it is scientifically suggestive that they are responsible for the antibacterial activity in many plants [20]. These findings are consistent with the reports of De et al. [21] of which 35 different Indian species and herbs indicated among others with potent antimicrobial activities against some test organisms like *B. subtilis*, *E. coli* and *Saccharomyces cerevisiae* [21,22]. Thus, phytochemicals present in aqueous leaves extract of *N. canescens* can be reported as being responsible for the biological activities

Table 2: Acute Toxicity Studies of the Extract *Nelsonia canescens* in Mice (Phase 1).

Plant	Parts used	Dose (mg/kg bw)	No of animals used	Mortality	Toxicity signs
Nelsonia canescens	Leaf	10	3	0	No observable sign of toxicity
Nelsonia canescens	Leaf	100	3	0	No observable sign of toxicity
Nelsonia canescens	Leaf	1000	3	0	No observable sign of toxicity

Table 3: Acute toxicity studies of the extract *Nelsonia canescens* in mice (Phase 2).

Plant	Dose (mg/kg bw)	No of animals used	Mortality	Toxicity signs
Nelsonia canescens	1500	1	0	Weak and inactive but become normal
Nelsonia canescens	1750	1	0	Inactive but became normal
Nelsonia canescens	2000	1	0	Shivering and inactive but became normal after 1 hour

of this plant as reported by other scientists and herbalists who use it traditionally for the treatment of wide array of illness including treatment of fever, pain, chickenpox, measles, constipation and gastric ulcer (Acharya et al 2012) [22]. In Africa, the plant is used to reduce fever and as an analgesic in a wide range of conditions including colds, flu, and also viral infections [23].

This work is also in agreement with who reported that aqueous leaf extract of *N. canescens* has therapeutic and antioxidant properties, that the phenolic compounds: tannins present in the leaf extract of *N. canescens* have the ability to cap the gold nano particles by ionic interaction and thereby stabilizing them [23,24]. Similarly, a number of researchers have linked the presence of certain phytochemicals in plants as being responsible for the successful treatment of specific diseases; Tannins and flavonoids are reported to be present in extracts used as antibacterial and antioxidant [25]. Flavonoids and glycosides are also known to prevent cardio-vascular diseases and ulcers [26]. The presence of alkaloids in many plant extracts is suggestive of their reasons for a wide range of pharmacological activities including anti-malaria, antiasthma, anticancer, etc., [27]. This work is in agreement with other scientific reports that due to the presence of many phytochemicals in this plant its aqueous extract could be used for curative activity against many pathogens and therefore explains the use of *N. canescens* by many traditionalists in Africa for the treatment of wide array of illness including malaria (Anaduaka et al., 2013).

Results from (Tables 2 and 3) of this study shows the acute toxicity test of the plant's aqueous extract of *N. canescens* is greater than 2,000 mg/kg which means the plant has a very wide safety margin even though the mice were shivering and became weak at higher doses but no death was recorded even after 24 hours. The Organization for Economic Cooperation and Development (OECD) guideline recommended chemicals labeling and classification of acute toxicity (LD50) based on oral administration as follows: very toxic substances with an LD50 ≤ 5 mg/kg, while toxic substances having an LD50 to be >5 mg/kg ≤ 50 mg/kg, on the other hand, harmful substances are said to have an LD50>50 mg/kg ≤ 500 mg/kg, and no label >500 mg/kg ≤ 2,000 mg/kg respectively (OECD, 2001). This work is in agreement with similar reports of other plants; reported that mice fed with up to 3,000 mg/kg body weight of the ethanolic extract of Anacardium occidentale (cashew) showed sign of weakness but later became active and the authors concluded that the plant extract is safe Sha'a et al. [28]. Reports of other toxicological studies show that dosage up to 5,000 mg/kg body weight of ethanolic extracts of *Newbouldia laevis*, aqueous leaf and root extracts of *Cymbopogon citratus* and crude hydroalcoholic extracts of *Embelia schimperi* are safe (Anaduaka et al. 2013). However, reported that ethanolic extract of flower of *Newbouldia laevis* is moderately toxic because the LD50 in mice was found to be 1,264.9 mg/kg body weight when administered through intra-peritoneal route [29-33].

Conclusion

In this present study, the aqueous leafs extract of *N. canescens* possesses a variety of phytochemical constituents, the results obtained from acute toxicity studies of the aqueous leafs extract of *N. canescens* showed that the aqueous leaves extract of *N. canescens* did not produce mortality, signs of toxicity and are relatively safe above 2,000 mg/kg body weight. The results of this study suggest that aqueous leafs extract of *N. canescens* can be considered a plant with natural antibiotics at doses used in this work, these scientific data justifies the traditional use of *N. canescens* for treatment of pain, reduce fever, inflammation, constipation and gastric ulcer. Further studies are recommended for possible identification of the active ingredients and Isolations of functional group present in the plant extract.

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