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EVALUATION OF ANTI-HELMINTICS EFFECT OF *NELSONIA CANESCENS* PLANT LAM. (ACATHECEAE)

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ABSTRACT
Natural products play an important role in the discovery and development of new and effective pharmaceuticals. In the present study, we assessed the antihelmintics properties of the leaves of *Nelsonia canescence*. The pulverized leaves were cold macerated in ethanol for 48 hrs, half of the crude extract was then fractionated using n-hexane, ethylacetate and butanol. Pharmacognostic profile, phytochemical analyses, and acute toxicity were determined using standard procedure. The ethanol extract and fractions were tested for antihelmintics activitiy. The antihelminthic tests were carried out using astandard procedure. Five petri dishes was labeled group 1 to 5 and three worms was introduced into each petri dish. Group 1 and 2 received 10 mls of 0.5 % CMC and 25 mg of Albendazole respectively, group 3 to 5 received 12.5, 25 and 50mg of the crude extract. The worms where observed for paralysis and or death. The mean time for paralysis was noted. The leaves were found to be dark green with acute apex, reticulate veination, and bitter taste. Phytochemical analysis revealed the presence of flavonoids, saponins and tannins. Proximate analysis of *N. canescense* revealed moisture content of 7.3 % and ash value of 19.5 %. The plant was relatively non toxic with $LD_{50} \geq 5000$ mg/kg. At the dose of 50mg/ml, *N. canescens* crude extract and ethylacetate fraction showed a significant ($p<0.01$) paralysis and the mean time of death was extremely significant ($P<0.01$). The study has shown that the leaf of *N. canescens* crude extract and ethylacetate fraction possesses antihelmintics activity.

KEYWORD: Antihelmintics, *Nelsonia canascens*, Phytochemicals, paralysis.

BACKGROUND
Helminthes infections are the most common parasitic infections in man which affects the large proportions of the world's population. Helminthes infestation causes morbidity and mortality. It compromises nutritional status, affects cognitive processes induces tissue reactions and cause intestinal obstruction or rectal prolapsed. For reasons not well understood, school-aged children (including adolescents) and pre-school children tend to harbor the greatest numbers of intestinal warms and shistosomes as a result, experience stunted growth and diminished physical fitness as well as impaired memory and cognition that leads to educational deficits.[1] These parasitic worms also infect the livestock and crops thus officially the food production with a result and economic impact. They includes the intestinal nematode (round worm) tremeloda (fluka) and cealodes (tape worms).[2] It comes as no surprise, that the drugs available for human treatment were first developed as veterinary medicine. In some cases, this situation has been exacerbated by the remarkable success of Ivermectin over the last twenty year throughout the world; the parasitic helmintic infection increases the mortality and morbidity day by day.

Anthelmintics are drugs that expel parasitic worms (helminths) from the body, by either striking or killing the worms.[3] They are also known as vermifuges or vermicides. Anti-helmintics are also known as the tropical and veterinary types of medicines which are of huge importance. The high medical, educational, and economic burden of helminth infections, together with their co-endemicity with malaria and AIDS, provides an important rationale for launching a global assault on parasitic worms. However, the tools we currently have for controlling worm infections are limited; of the 1,556 new chemical entities marketed between 1975 and 2004, only four drugs — albendezole, oxamniquine, praziquantel, and ivermectin — were developed to treat helminthiasis. Currently, the control of parasitic helminths relies chiefly on the use of synthetic, broad-spectrum anthelmintic drugs. In all, major classes

anthelmintics commercially available include benzimidazoles, macrocyclic lactones.[4] However, inappropriate and exclusive application of these drugs has contributed to the development of extensively drug resistanct parasites. There is an increase in resistance to anti-helmintic drug especially in nematode species which affect human beings, sheep, horses, cattle and pigs.[1] Additionally, treating with already existing ant-helmintic drugs has led to several side effects where the individual reported, allergy, nervous system symptoms, gastrointestinal disturbances and allergic phenomena. Antihelminthics are also known to produces toxicity in human beings. Hence, the development and discovery of new substance acting as antihelminthics are being derived to be the best source of bioactive substances. The origin of many effective drugs is found in traditional medicine practices has made several researchers to undertake studies for evaluating folklore medicinal plants on their proclaimed anthelmintic efficacy hence, medicinal plants practiced in folk medicine can serve as a source of affordable and effective antihelmintics agents.[5] 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.[6]

The plant *Nelsonia canescense* which belongs to Acanthaceae family is used as an anthelmintic agent as well as for common application in folk medicine. *N. canescens* is used in African and Asian traditional medicine. 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.[7] In India, it is called "Bada Rasna" and it is used in traditional medicine to treat pain and inflammation.[8] *N. canescens* is also reported to be used as a cover crop to suppress the growth of weeds in banana plantations. Here this species can invade large areas of the plantation with no visible adverse effects on the banana crop but limiting the possibility of other weeds to invade.[9] *N. canescens* was also reported to have analgesic and anti-inflamatory propreties.[10] *N. canescens* have been used for a long time in diverse contexts, i.e. as an ornamental plant, antioxidant[11], antibacterial, anti-inflammatory, analgesic, purgative, and antispasmodic.[12] *Nelsonia canescens* 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.[13]

METHODS

Chemicals

Albendazole (Bendex) obtained from Cipla company, other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Experimental animals

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in vitro. Earthworms were collected from the damp soil at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka and washed with normal saline to remove all the faecal matter. *P. posthuma* with 6-8 cm in length and 0.3-0.5 cm in width was used for anthelmintic activity test. The worms were identified in the Department of Zoology, Faculty of Biological Sciences, Nnamdi Azikiwe University Awka. This organism was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.[14] Before initiation of experiment the earthworms were washed in normal saline.

Adult Swiss albino rats (190-230 g) was also used for the study and was obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University. The animals were fed with palletized feed (UAC feed, Nigeria) and had access to water *ad libitum.* Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. They were allowed free access to food and water. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub. No. 85 – 23 Revised 2011).

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

Plant collection and identification

Plant material: *Nelsonia canescences* plant were collected from Agulu in Anaocha local Government area Anambra state Nigeria. It was authenticated by a trained taxonomist, Mr Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimens (No. PCG 894/A/006) were deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka for future reference. The plant materials were subsequently cleaned, the fruit pulp separated from the seeds while the roots were shredded then air-dried at room temperature for 3 weeks and pulverized with a mechanical grinding machine (GX160 Delmar 5.5HP).

Method of Extraction

Five hundred grams (500 g) of the pulverized *Nelsonia canescense* plant was macerated in one liter of ethanol over a period of 48 hours. The mixture was sieved using porcelain cloth. It was further filtered with no.l Whattman filter paper. The filtrate was concentrated using rotary evaporator. It was further dried in a water bath at a temperature of 40° C to obtain the crude extract. The extract was then stored in a refrigerator for use.

Fractionation (Liquid-liquid chromatography)
The methanol extract (100 g) was subjected to liquid-liquid containing water (200 mL in 100 g of extract) were subjected to liquid-liquid partition successively with 1000 ml n-hexane, 1000 ml ethyl acetate and 500 ml n-butanol in increasing order of polarity. The fractions were filtered with Whatman no 1 filter paper and concentrated *in vacuo* using rotary evaporator at 40 °C to obtain the n-hexane fraction (HF), ethylacetate fraction (EF) and butanol fraction (BF). The extracts and all the fractions were stored in refrigerator between 0-4°C until they were used.

Macroscopic evaluation
Organoleptic evaluation of the powdered plant was carried out using standard methods.[15] Sensory organs were used to evaluate the colour, odour, taste and texture of the plant powder.

Phytochemical analysis
The phytochemical screening was carried out on the crude extract and fractions of *N. canescence* leaves according to standard methods to identify the classes of bioactive compounds present.[15,16]

Acute-toxicity and lethality (LD_{50}) test
Acute toxicity analysis of the extracts was performed using Lorke's method.[16] Thirteen(13) Winster albino rats were utilized in this study. The test involved two stages. In stage one: the animals were grouped into three (3) different groups of three rats each. They were administered 10, 100, and 1000 mg/kg body weight respectively and in the second stage, 1600, 2900 and 5000 mg/kg body weight of the extract were administered. The administration of the extract was done orally. The LD_{50} was calculated using the formula:

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

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Anthelmintic activity on Pheretima posthuma
The anthelmintic activity was performed according to the method followed by Shelke *et al* (2020). All the experiments were carried out in Nigerian adult earthworms due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. They were collected from moist soil and washed with normal saline to remove all fecal matters. Five Petri-dishes were labeled group I-V. Group I contains 10 ml of 0.5 % CMC, group II contains 10 ml of 20 mg/ml albendazole. Group III contains 10 ml of 12.5 mg/ml extract. While group IV contains 10 ml of 25 mg/ml extract, while group v contains 10 ml 50 mg/ml extract. Each petridish was placed with 3 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. Also the weights of the worms were taken before and after death. The test drug results were compared with control group and reference compound (albendazole) treated group.

Statistical analysis
The results are presented as mean±SEM (Standard Error of Mean). Statistical analyses graphical representations of results for anthelmintic study were evaluated by one-way ANOVA following Dunnett's Multiple Comparisons test using Graph Pad Prism version 8.4 for windows. Graph Pad Software, San Diego California USA, the $p<0.005$ was considered to be statistically significant.

RESULTS

4.1 Extraction yield
The extraction yielded 10g of the crude extract and with extractive value of 2.5% from which the different doses used for the test was prepared by dissolving in ethanol

4.2 Macroscopic examinations of the whole leaves
The result of the macroscopic examination of the whole leaves of *N. canenscens* as shown in Table 1.

Table 1: Macroscopic examination of *N. canenscens.*

Macroscopic examination	Observations
Colour	Dark green
Margin	Entire margin lamina
Apex	Acute
Venation	Reticulate
Size	Up-right, up to 20cm tall
Texture	Rough
Surface	Glabrous
Odour	Characteristic
Taste	Bitter odour

The phytochemical screening revealed that ethanol extract contains terpenoids, proteins, carbohydrates resins, flavonoids, tannins, glycosides and saponins.

Table 2: The result of the phytochemical screening of *N. canescense* powder is presented on table below.

s/n	Test	Presence
1	Alkaloids	_
2	Saponins	+
3	Tannins	+
4	Flavoniods	+
5	Steroids	-
6	Terpeniods	+
7	Cardiac glucosides	+
8	Carbohydrates	+
9	Proteins	+

Key: - =absent; +=present

Result of the acute toxicity of ethanol extracts of N. *canescense* leaf

The fasted animals used in the first phase of the test were observed to be visibly calm after oral administration. No visible signs of pain or discomfort were observed. From the toxicity study, it was observed that the powdered leaves of N. *canescense* leaf was non-toxic and caused no death up to 5000mg/kg orally. Toxicological studies established LD_{50} of the crude to be greater than 5000mg/kg showing it is safe for consumption.

Result of anthelmintic activity

The crude extract and factions of *N. canescense* produced a significant (p<0,01) anthelmintic activity in dose dependent manner as shown in Table 4. Both crude extract (CE) and fraction (ethyaceatae fraction EF, butanol fraction BF and n- hexane fraction NF) took less time to cause paralysis and death of the earthworms as compared to aqueous extract. At 12.5mg/ml of ethyl acetate the time of paralysis was moderately significant (97 %, p<0.01) and the time of death was insignificant (94 %, p>0.05). At 25mg/ml of ethyl acetate the time of paralysis was moderately significant (97%, p<0.01) and the time of death was also moderately significant (95 5%, p<0.01).At 12.5mg/ml of N-hexane the time of paralysis was moderately significant (85 %, p<0.01) and the death time was significant (83 %, p<0.01). At 25mg/ml of N-hexane the time of paralysis was significant (88 %, p<0.01) and the time of death was also significant (88 %, p<0.01). At 12.5mg/ml of Butanol the time of paralysis was significant (94 %, p<0.01) and the time of death was also significant (89 %, p<0.01). 25mg/ml of Butanol fraction was significant (94 %, p<0.01) at the time paralysis and the time of death was also significant (95 % %, p<0.01).

Table 3: Effect of *N. canescense* crude extract and factions on earthworms.

Group	Mean Time of paralysis	Mean Time of death	Initial weight	Final weight	% mean death time	% mean paralysis
50mg/ml CE	0.17±0.05****	0.56±0.18 ****	3.07± 0.009	2.52± 0.00	95	98
25mg/ml CE	0.32±0.02****	1.07±0.12 ****	1.60± 0.04	0.5± 0.006	92	96
12.5mg/ml CE	0.87±3.83 ****	2.00±0.21 ****	0.95± 0.01	0.87±0.06	85	91
20mg/ml Albendazole	2.32±0.04 ****	3.68±0.26 ****	1.02± 0.05	0.86±0.04	72	77
0.5% CMC	10.32± 0.57	13.45±0.86	1.10±0.04	1.12±0.02	0	0
12.5mg/ml EF	0.26±0.01 ****	0.78±0.04 ****	1.4±0.58	1.86±0.32	94	97
25mg/ml EF	0.14±0.01 ****	0.48±0.03 ****	1.22±0.43	1.44±0.47	95	97
12.5mg/ml HF	1.53±0.28 ****	2.22±0.9 ****	0.84±0.39	1.20±0.29	83	85
25mg/ml HF	1.21±0.06 ****	1.57±0.08 ****	0.84±0.39	1.20±0.29	88	88
12.5mg/ml BF	0.53±0.06 ****	1.37±0.08 ****	1.02±0.44	1.23±0.43	89	94
25mg/ml BF	2.32±0.04 ****	0.94±0.07 ****	0.96±0.39	1.26±0.42	95	94

All the values are expressed as mean ± SEM, N=3). *p < 0.05, ****p < 0.01 as compared with Control group (one way ANOVA followed by Dunnett t-test, 2 sided).

DISCUSSION

The macroscopic examination of the whole leaves of *N. canenscens* showed that it has an entire margin lamina, acute apex, reticulate venation and the glabrous surface. The organoleptic property reveals that the front surface of the leaf is dark green in colour and it has a bitter taste. Macroscopic techniques may be useful to discriminate morphologically similar plant to distinguish between the desired plant species and plant part in the field during the plant sampling. According to World Health Organization, the Macroscopic determination of plants is the first step towards establishing its identity and purity hence, should be carried out before any test is undertaken.[18]

The percentage yield of the extraction process of *Nelsonia Canescense* was 2.5%. The extractive values are relevant when the chemical natures of the medicinal components are not known and the values help in extraction procedures.[19]

The oral median lethal dose (LD50) was estimated to be ≥5000mg/kg body weight, the absence of death following the oral administration of the crude extract of *Nelsonia canescense* at 5000 mg/kg body weight observed in the rats suggest that the extract is practically non-toxic acutely.[20]

The results of the present study has shown that the leaf of *N. canescense* showed a dose dependent significant (p<0.01) antihelmintics activity. The crude extract at all doses (12.5, 25 and 50 mg/ml) has a significant (p<0.01) mortality rate and percentage paralysis with percentage mean death time and paralysis of 85 and 91% for 12.5mg/kg, 92 and 96% for 25mg/kg and 95 and 98% for 50mg/kg compared to the positive control (Albendazole 72 and 77%). Albendazole is known to cause paralysis of worms so that they are expelled in faeces of man and animals. The crude extracts not only demonstrated this property, but they also caused early death of worms at all concentrations. The fractions exhibited a dose dependent significant (p<0.01) antihelmintics activity with ethylacetate fraction giving the highest activity. At

12.5mg/ml of ethyl acetate the time of paralysis was moderately significant (97 %, $p<0.01$) and the time of death was insignificant (94 %, $p>0.01$). The above findings justify the anthelmintic properties of *N. canescense* and the extent of activity depends on the phytochemical constituent present in the extract or fraction.

This study revealed anthelmintic activity of both the crude extract and fractions and their this activities are described for the first time. Delaquis *et al*[21] observed naturally occurring combinations of plant compounds showed synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents. N. canescense could be made part of an integrated management plan for control of helminths in developing countries like Nigeria.

CONCLUSION

In this study, *Nelsonia canascens* plant has been proven to possess antihelminthic activity and. Further studies are required to isolate and reveal the active compound contained in the crude extracts to establish the mechanism of action.

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Conflict of interests

Declared none

REFERENCES

1. Crompton DW, Nesheim M.C. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu. Rev. Nutr., 2002; 22: 35–59.
2. Robinson MW, Grieg R, Beattie KA, Lamont DJ, Connolly B. Comparative analysis of the excretory-secretory proteome of the muscle larva of *Trichinella pseudospiralis* and *Trichinella spiralis*. Int. J. Parasitol, 2005; 37: 139–148.
3. Yashaswini T, Akshara KA, Prasad K, Kumar MD, Akshaykumar K. Anthelmintic activity of seed extracts of *Artocarpus heterophyllus*. IOSR J Pharmacy Biolog Sci., 2016; 11(5): 19-23.
4. Kaplan RM, Vidyashankar AN. An inconvenient truth: Global worming and anthelmintic resistance. Veterinary Parasitology, 2012; 186(1–2): 70–78.
5. Ekeanyanwu RC, Etienajirhevwe OF. In vitro anthelmintic potentials of Xylopia aethiopica and Monodora myristica from Nigeria. Afr J Biochem Res., 2012; 6: 115-120.
6. Saganuwan AS. Some medicinal plants of Arabian Peninsula. African. Journal of Microbial, 2010; 4(9): 766-788.
7. McDade LA. Toward a comprehensive understanding of phylogenetic relationship among lineages of Acanthancea S. L(Lamiales). American Journal of Botany, 2008; 95(9): 1136-1152.
8. Acharya S, Dash GK, Brahma DK, Chhetree R. R. Preliminary pytochemical investigation and anthelmintic activity of *Acacia suma* (Roxb) barks. IRJP, 2011; 2(1): 136-141.
9. Fongod AGN, Focho DA. Mih AM. Fonge BA. Lang PS. Weed management in banana production: the use of Nelsonia canescens (Lam.) Spreng as a non-leguminous cover crop. African Journal of Environmental Science and Technology, 2010; 4(3): 167-173.
10. Bamidele O, Yusuf YO, Elizabeth AB, Ayodele OO. Analgesic and anti-inflammatory properties of Nelsonia canescens leaf extract. Journal of Ethnopharmacology, 2005; 99(1): 153-6.
11. Sawadogo WR, Meda R, Lamien CE, Kiendrebeogo M, Guissou IP, Nacoulma OG. Phenolic content and antioxidant activity of six Acanthaceae from Burki. Journal of Ethnopharmacology, 2006; 99(1): 153-6.
12. Focho DA, Ndam WT, Fogne, BA. "Medicinal plants of Aguambu-Bamumbu in the Lebialem highlands, southwest province of Cameroon" African Journal of Pharmacology, 2009; 3(1): 001-013.
13. McDade LA. Phylogenic placement, delimitation, and relationships among genera of the enigmatic Nelsonsonia (Lamiales: Acanthaceae). Taxon, 2012; 61(3): 63–76.
14. Shelke PS, Jagtap PN, Tanpure PR. In-vitro anthelmintic activity of Boswellia serrata and Aloe barbadensis extracts on Pheretima posthuma: Indian earthworm. Int J Res Med Sci., 2020; 8: 1843-7.
15. Evans WC. Trease & Evans pharmacognosy. 15th Edn. W. R. Saunders, London, 2002; 214-393.
16. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan, Nigeria, 1993; 1: 153. 21.
17. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology, 1983; 54(4): 275-87.
18. Khandelwal KR. Practical pharmacognosy techniques and experiment.17th edition, Pune: Nirali, Prakashan, 2007.
19. Jegede IA, Ibrahim JA, Kunle OF. Phytochemical and pharmacognostic studies of the leaf of stem bark of Anthocleista vogelil (Planch). Journal of medicinal plant research, 2011; 5(26): 6136-6139.
20. Mbagwu, IS, Akah PA, Ajaghaku, DL. *Newbouldia laevis* improved glucose and fat homeostasis in a type 2 diabesity mice model. JEthopharmacol, 2020; 6,251: 112555.
21. Delaquis PJ, Stanich K., Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol, 2002; 74: 101-109.