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Adeleke kolapo
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Bernard Omomoh
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Nwachukwu Claret
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Oparanozie Barbara
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Ori Kelechi
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Osemenam Azuka
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Osemenam Uche
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Osemenam Chidima
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Osemenam Ifeanyi
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Correspondence
Bernard Omomoh
Department of Pharmacognosy,
Madonna University Elele
Campus, River States, Nigeria

Evaluation of antihelmintic properties of methanol stem bark extract of *Khaya grandifoliola* (Meliaceae)

Adeleke Kolapo, Bernard Omomoh, Nwachukwu Claret, Oparanozie Barbara, Ori Kelechi, Osemenam Azuka, Osemenam Uche, Osemenam Chidima and Osemenam Ifeanyi

Abstract

This experiment deals with the evaluation of the antihelmintic properties of methanol stem bark extract of *Khaya grandifoliola*. *Lumbricus terrestris* (earth worms) were placed in different concentrations of 25, 50, 100, 200 and 400 mg/ml of *Khaya grandifoliola* methanol extract and 10, 20, 40 and 80 mg/ml of n-hexane, chloroform, ethyl acetate and methanol-aqueous fractions in separate petri-dishes for a maximum of 3hrs. The time for death and paralysis of the organisms were noted at intervals of 30 minutes. Distilled water was used as control while Levamisole and Mebendazole (5 mg/ml) each served as reference standard.

Keywords: *Lumbricus terrestris*, 5mg/ml mebendazole, 5mg/ml Levamisole, *Khaya grandifoliola*

1. Introduction

Man in his attempt for survival, has engaged in several activities; some which exposes him, to several conditions. A typical example of such exposure is the exposure to worms (helminthis). The word 'helminthes' is derived from the Greek word *helminis* meaning worm. Specific types of helminthes, tend to cause certain harm to man, they deprive man of food, injure his organs, provide entry for other organisms Hence they are referred to as parasitic worms^[1]. They include, *Ascaris*, *lumbriocodes*, *schistosoma spp.*, *Necator americanus*, etc. Man, in order to survive, develops means to combat this, worms; they are known as antihelmintics. Anthelmintics ranges from the use of various classes of drugs: herbal to synthetic ones. This drug approach is one of the several practices commonly used, other includes; proper hygiene maintenance during cooking of foods, wearing of good safety guards during environmental sanitation, good sewage disposal^[2]. This study is aimed at evaluating the antihelmintic properties of *Khaya grandifoliola*, as a herbal approach and comparing its efficacy to the modern synthetic drugs use nowadays.

2. Materials and method

2.1 Plant collection

Plant was collected in Akure, Ondo state, Nigeria. It was identified and authenticated by Bernard Omomoh at Obafemi Awolowo University, Ile-Ife, Nigeria on 16th April, 2015 with herbarium number 17459 after collection by Osemenam Henry on 15th April, 2015. A Voucher specimen was deposited at both Department of Pharmacognosy OAU and Madonna University, Nigeria. The bark of the plant sample was chopped and dried. The bark was now grinded to a coarse powder and later stored in a plastic container prior to extraction.

2.2 Plant extraction

The grounded crude drug (2kg) was macerated with 6,500ml of methanol for 72 hours, the solution was decanted, filtered, and separated from the marc. This filtrate was concentrated using a rotary evaporator to reduce its volume. It was later placed on a water bath at a reduced temperature (40 °C) to evaporate the solvent completely. The dried extract was then transferred to a glass bottle and stored in desiccators, prior to use. 80g of the dried extract was solvent-partitioned separately into the various compartments based on their solubility properties (n-hexane, chloroform, ethyl acetate and methanol) and the resulting fractions were kept for further assay.

2.3 Animals

Species of *Lumbricus terrestris* worms (10cm-14cm long by 0.2-0.4cm width) were collected in damp and muddy areas of Madonna University campus, Elele, Rivers State and identified at

Zoological Department, University of Ibadan, Nigeria. The earth worms were divided into eight groups of six animals each in a separate petri-dish and exposed to different concentrations of the methanol extract (group I, II, III, IV, V and VI were exposed to distilled water 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml respectively) while group VII and VIII were exposed to 5mg/ml Levamisole and mebendazole respectively. Each test was repeated in triplicate and paralysis and death of the animals were examined at 30 minutes intervals. Another groups of earth worms of six animals each were also used to evaluate n-hexane, chloroform, ethyl acetate and methanol aqueous fractions (10, 20, 40 and 80 mg/ml) each for their antihelminthic activity.

2.4 Phytochemical Evaluation

This was carried out according to department of pharmacognosy, UI, Practical manual, Sofowora, Trease and Evans. Preliminary phytochemical screening was performed on the methanol extract of the powdered plant sample for tannins, flavonoids, anthraquinone, saponin, steroids, Glycosides and alkaloid as follows [3, 4, 5].

2.4.1 Test for Glycosides

About 0.2g of the extract was hydrolyzed with HCL and the mixture neutralized with NaOH solution. Fehling solution A&B were added and the mixture heated. Absence of precipitate was taken as absence of glycosides

2.4.2 Test for saponin

About 20ml of distilled water was added to 2.0g of the powdered sample and boiled on a hot water bath for 2 minutes and filtered. The filtrate was allowed to cool and then used for the following tests

- (a) **Frothing test:** About 5ml of the filtrate was diluted with 15ml of distilled water and shaken vigorously. Formation of stable froth indicates the presence of saponins
- (b) **Emulsion test:** About 2 drops of olive oil was added to 10ml of the frothing solution and the contents shaken vigorously. The formation of emulsion indicates the presence of saponins

2.4.3 Test for tannins

The powdered sample (2.5g) was boiled with 20ml of water, filtered and used for the following test

a) Ferric chloride test

To 3ml of the filtrate, few drops of ferric chloride were added. Formation of a greenish black precipitate indicates the presence of tannins.

b) Lead acetate test

To 3ml of the filtrate was added lead acetate solution. Formation of precipitate indicates the presence of tannins

2.4.4 Test for flavonoids

The extract (2g) was dissolved in dilute NaOH to give a yellow colored solution. Addition of dil HCL turned the solution colorless, indicating the presence of flavonoids.

2.4.5 Test for steroids

Acetic anhydride (2ml) was added to 0.2g of the extract, followed by addition of 2ml H₂SO₄, the changing of color from violet to green was taken as indication for presence of steroids.

2.4.6 Test for alkaloids

The powdered sample (1g) was dissolved in 5ml of water, heated on a boiling water bath for 10 minutes, cooled and filtered with what man filter paper. It was tested with a pH metre to ensure it was at pH of 6. It was dived into five (5) test tubes 1ml each; filtrate was tested with a few drops of Mayer's reagent (Potassium mercuric iodide solution), Dragendorff's reagent (Bismuth potassium iodide solution), Wagner's reagent (iodo- potassium iodide solution), Picric acid solution (1%) and 10% tannic acid solution. A creamy yellow precipitate was formed with Mayer's reagent; creamy yellow precipitate with dragendorff's reagent. A yellowish cloudishsolution was formed with (1%) picric acid solution and a brownish precipitate was formed with (10%) tannic acid solution, indicating the presence of alkaloids.

2.4.7 Test for anthraquinone

(a) **Test for combined anthraquinone:** The powdered sample, was weighed (1g) and 10ml of 10% conc. HCL was added. An addition of 90ml of water was added to this mixture; it was separated into two equal volumes of chloroform (highly coloured and low coloured volumes) and 2ml 10% ammonia was added to the less coloured fraction. Absence of a brown ring indicated the absence of combined anthraquinone.

(b) **Test for free anthraquinone:** The powdered sample was weighed (0.5g), 5ml of 10% chloroform was added to this sample, 2ml of 10% ammonia was added, absence of a brown ring indicated absence of free anthraquinone.

2.5 Biologocal activity: Anthelmintic assay

The different concentrations of the extract were placed in separate petri- dishes and then the antihelminthic drugs (Mebendazole and Levamisole) were used as standard while distilled water was used as control. The worms were exposed to distilled water, the extract (at different concentrations) and standards, and then observed for paralysis and death at 30 minutes intervals. The solvent- partitioned fractions (n-hexane, chloroform, ethyl acetate and methanol / aqueous) were also subjected to antihelmintic assay as above [6].

2.6 Data analysis

All the data were subjected to statically analysis using ANOVA.

3. Results and discussions

Table 1: Phytochemical analysis of *Khaya grandifoliola* stem bark

Phytochemical screening of the crude sample powder of *Khaya grandifoliola* shows the presence of Alkaloids, Tannins, flavonoids, Saponins, Reducing sugar and Steroids.

Tests	Results
Alkaloids	+
Tannins	+
Saponins	+
Anthraquinones	-
Reducing sugar	+
Flavonoids	-
Glycosides	+
Steroids	+

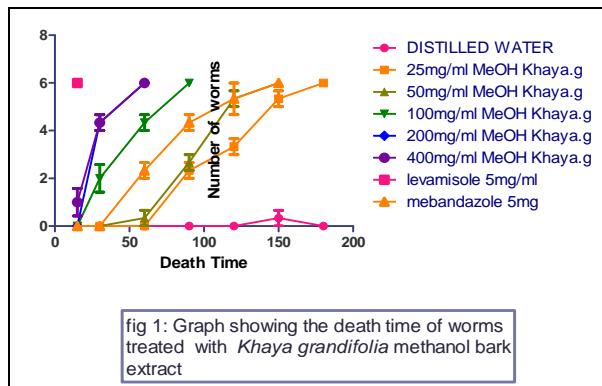


fig 1: Graph showing the death time of worms treated with *Khaya grandifolia* methanol bark extract

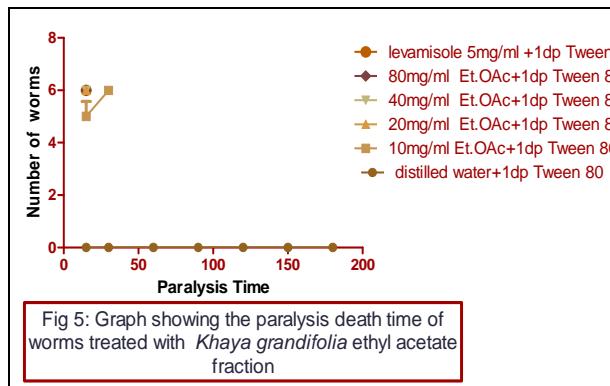


Fig 5: Graph showing the paralysis death time of worms treated with *Khaya grandifolia* ethyl acetate fraction

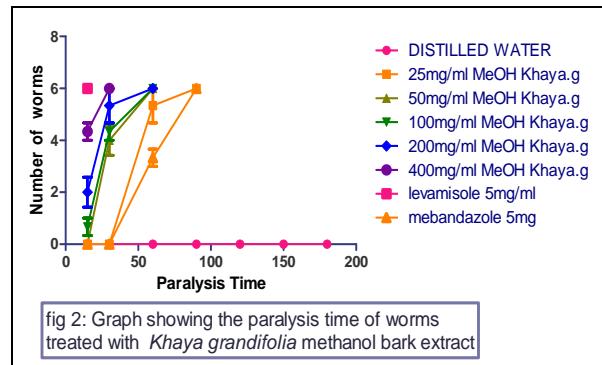


fig 2: Graph showing the paralysis time of worms treated with *Khaya grandifolia* methanol bark extract

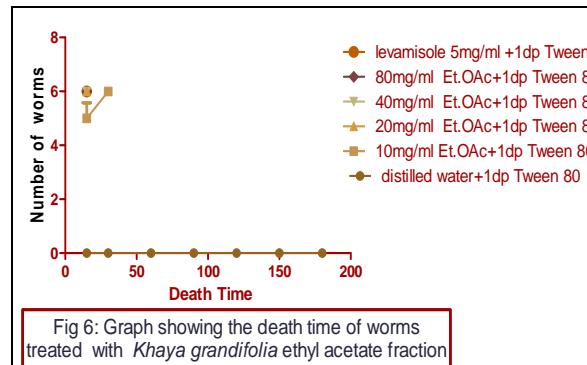


Fig 6: Graph showing the death time of worms treated with *Khaya grandifolia* ethyl acetate fraction

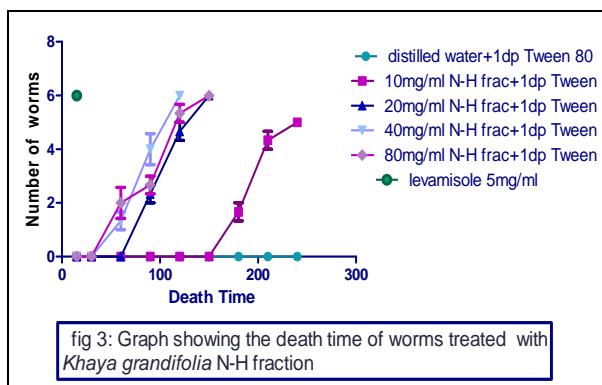


fig 3: Graph showing the death time of worms treated with *Khaya grandifolia* N-H fraction

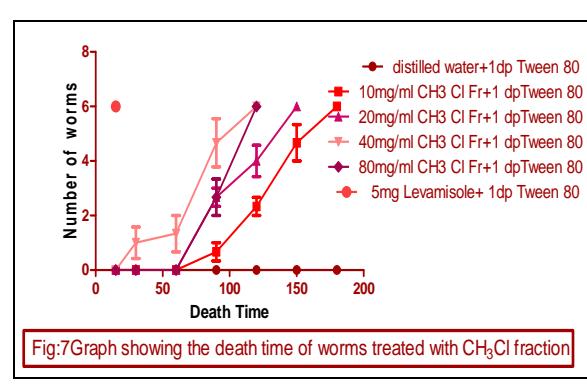


Fig 7: Graph showing the death time of worms treated with CH₃Cl fraction

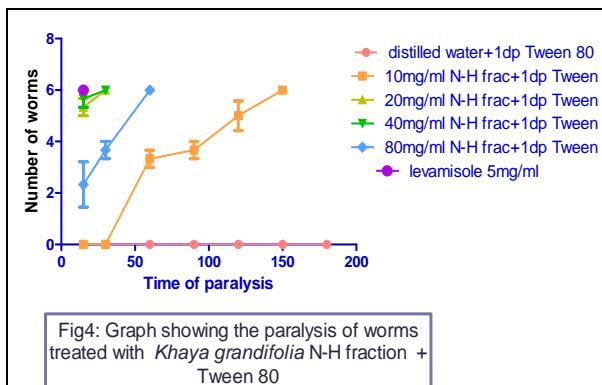


Fig 4: Graph showing the paralysis time of worms treated with *Khaya grandifolia* N-H fraction + Tween 80

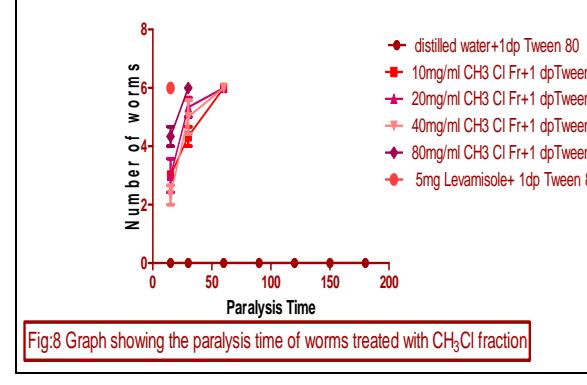
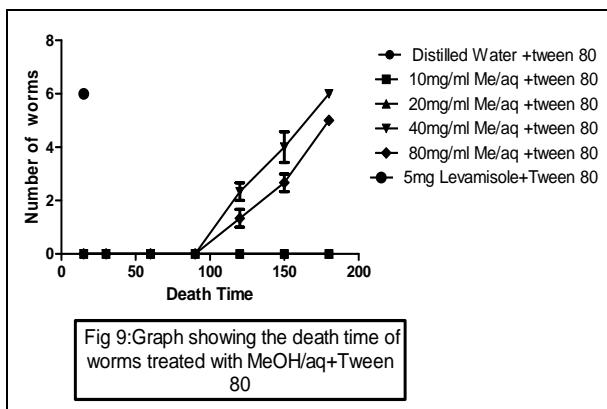
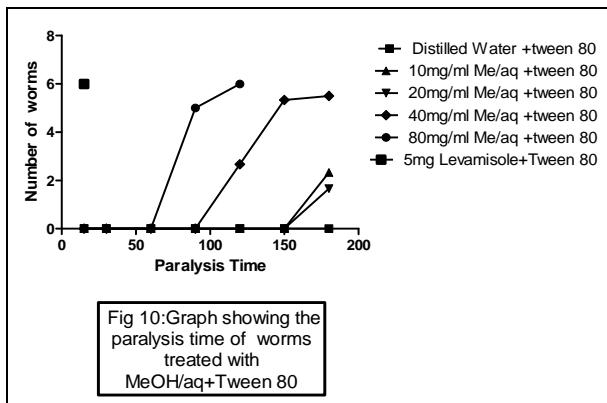


Fig 8: Graph showing the paralysis time of worms treated with CH₃Cl fraction



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4. Conclusions

The crude extract at 200mg/ml, 400mg/ml and the ethyl acetate fraction (80 mg/ml+ tween 80) had a 100% antihelminthic activity in comparison with the standard (levamisole 5mg). They all killed the worms in 30minutes. These concentrations may serve as a research point for researchers in the manufacture of a new antihelminthic drug. The most effective fraction is the ethyl acetate fraction as it killed the worms in 30mins. This is similar to the work of Bachaya *et al.*, 2009, as the activity increases with time against concentrations [7]. 10mgf of all the fractions produced low death compared to all other concentrations, this might be a pointer that the extract from this drug might not have a narrow therapeutic margin when used in the production of drug.

5. Acknowledgement

Almighty God, thanks.

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