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Growth inhibitory and cytotoxic effects of the methanol extract of *Brachystegia eurycoma* Harms (Fabaceae) leaves

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Abstract

Brachystegia eurycoma Harms (Fabaceae) is used Nigeria for its anti-inflammatory, anti-malaria, anti-diabetics, and antihypertensive properties in combination with other plants. The seeds have particularly served as thickener in certain soups like '*egusi'* and '*ogbono'* in the eastern part of Nigeria. The growth inhibitory and cytotoxic effects of the methanol extract of the leaves and its organic solvent fractions were evaluated on the radicles of guinea corn *Sorghum bicolor* seed and tadpoles of *Raniceps ranninus* respectively. The extract and the aqueous fraction completely inhibited the germination of the guinea corn seeds in 24 h at a concentration of 20 mg/ml. The lengths of the radicles produced were significantly (<0.05) reduced with increase in the concentration of the extract and the fractions. The extract had an IC_{50} of 5 mg/ml while the aqueous fraction was observed to have 1.61 mg/ml. The cytotoxic effects of the tested extract and its fractions were observed to be concentration and time related. The aqueous fraction produced 100 % mortality at the concentration of 100 µg/ml with LC_{50} of 30 µg/ml whereas the extract produced 96.67 ± 3.33 % mortality at 200 µg/ml with LC_{50} of LC_{50} of 62.5 µg/ml. In conclusion, the results obtained confirmed the probable use of *B. eurycoma* leaves in treating tumour related ailments and the activities are enhanced with partial purification with the aqueous fraction showing higher activities.

Keywords: Brachystegia eurycoma, Growth inhibitory effects; Cytotoxic.

Introduction

Cancer characterized by uncontrolled proliferation of cells is one of the emerging prevailing ailments whose treatment involves the use of chemotherapeutic agents. Effective as some of the drugs may seem, their applications are sometimes plagued with non selectivity in their actions, high costs, non availability and serious undesirable side effects. For these reasons, search for medicinal plants with used in ethnomedicine in treating tumour related ailments becomes imperative particularly in developing countries where there is gross uneven distribution of health care facilities with appropriate man power (Steyn and Walker, 2000).

Locally called Ako in the Western parts of Nigeria, *Brachystegia eurycoma* Harms (Fabaceae) is one of the plants reportedly used in treating tumour related ailments. It is tree of about 35 m tall with bole of 2m diameter, vaguely buttressed, low branching, and large flat crown. It is common in river bank of the forest zone in southern Nigeria and Cameroon (Burkill, 1985). The

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leaf, bark and root of the plant are used ethno medicines in combination with other plant part in treatment of various diseases including malaria, diabetes, rheumatism, hypertension, and in bone setting (Adikwu *et al.*, 2007). In the eastern part of Nigeria, its seeds are used as thickener in preparation of local soups particularly '*egusi*' and '*ogbono*' soup (Keay *et al.*, 1964) while their wound healing and inflammatory properties have been mentioned in literature (Uhegbu, 2009). The ethanol and aqueous extracts of the bark have been reported to have antimicrobial activities particularly against fungi (Kunle, 2000). Apart from examining the phytochemical groups of constituents in the leaves, this work investigated the claimed use of the plant part in treating tumour related ailments, using two models - growth length of guinea corn *Sorghum bicolor* seed radicles and the extent of their toxicity to the tadpoles of *Raniceps ranninus*.

Experimental

Collection and preparation of the plant material. The leaves of *B. eurycoma* were collected from Iwo Osun State, and were authenticated by Dr. O. Shasanya, Taxonomist, Forest Research Institute of Nigeria, Ibadan where a herbarium specimen number FHI108436 was deposited. The leaves were dried in the oven maintained at 50^0C for four days. They were later reduced to powder form using a milling machine (Christy Norris, England). The powdered sample was kept in a closed container until needed.

Extraction of plant material. About 400g of the plant material was macerated with 1.5 L of methanol for 72 hours followed by filtration. The filtrate was concentrated to dryness under pressure using a rotary evaporator maintained at 40 ^{o}C. The concentrated extract obtained (29.22g representing 7.3%) was kept in a refrigerator until needed.

Organic solvent partitioning of the methanol extract. About 12g of the extract was dissolved in 50% methanol and partitioned repeatedly (3 X 200ml) with chloroform. After concentration, the chloroform and aqueous fractions (2.9 and 7.26 g respectively) were kept for further work.

Determination of growth inhibitory effects of the methanol extract and the organic solvent fractions on guinea corn (Sorghum bicolor). Guinea corn seeds obtained from Uselu market, Benin City were cleansed with absolute alcohol and air dried before use. The viability of the seeds was determined by their ability to sink in water. Those that remained submerged in water were removed and dried for use. 10ml of 0.5, 1, 2, 5,10 and 20 mg/ml of the methanol extract containing 5% Dimethyl sulphoxide (DMSO) in water was poured into 9 cm wide Petri dishes laid with cotton wool and filter paper (Whatman No 1). Twenty (20) viable seeds were spread on each. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 72, and 96 hours. The control seeds were only treated with 5% DMSO in distilled water containing no extracts. The procedure was repeated for both the chloroform and aqueous fractions obtained from the partitioning process of the methanol extract. The experiments were carried out in triplicates while the Statistical analyses were carried out using Analyses of Variance (ANOVA).

*Determination of cytotoxic effects of the methanol extract and the organic solvent fractions on tadpoles (Raniceps ranninus).*Tadpoles were harvested from small water settlements around the Faculty of Pharmacy, University of Benin and after proper identification by Dr. M. Aisien (Animal Parasitologist, Department of Animal and Environmental Biology, Faculty of Science, University of Benin), 5-7 day old tadpoles were removed and separated from others.

For the experiment, ten (10) tadpoles were selected with the aid of tube of Pasteur pipette (whose narrow end was removed) into 50ml beakers containing 15ml of the water from the source of the tadpoles which was made up to 49ml with distilled water. The volume was made up to 50ml with 0.5, 1, 2,5 and 10 mg/ml each of the extract dissolved in 5% DMSO in water to eventually obtain concentrations of 10, 20, 40, 100 and 200 µg/ml respectively. The procedure was repeated using the chloroform and aqueous fractions obtained from the partitioning process stated above. The controls were only treated with 1 ml of 5% DMSO while the tests were carried out in triplicates.

Results and Discussion

The methanol extract and the aqueous fractions remarkably reduced the germination rates of the guinea corn seeds compared with the controls. In 24 h, 65±2.9% of the controls germinated while the seeds treated with 10 mg/ml produced 32±6.0 % germination. At 20 mg/ml, both the extract and the aqueous fraction completely inhibited the germination of the seeds while the chloroform fraction produced 45±7.6 % germination at the same concentration (Fig. 1).

The extract and the fractions exhibited significant ($p<0.05$) concentration dependent reductions in the length of the radicles over the controls. While the average length of the radicles at 24 hours in the control seeds was 1.63 ± 0.94 mm, the average length in the seeds treated with 10mg/ml and 20mg/ml of the extract were 0.32 ± 0.06 and 0 ± 0 mm respectively. After 96 h incubation period, the average length of the control seeds was 9.43 ± 1.85 mm, whereas the seeds treated 10mg/ml and 20mg/ml of the extract showed average lengths of 0.58 ± 0.12 mm and 0.13 ± 0.08 mm respectively (Fig.2). The aqueous fraction was observed to be more effective than the chloroform fraction. At the end of 96 h, the control seeds produced radicle length of 12.43 ± 0.39 mm and this was reduced to 1.35 ± 0.24 and 0.3 ± 0.15 mm in seeds respectively treated with 10 and 20 mg/ml of the aqueous fraction, whereas the length of 34.05 ± 0.15 observed in the controls for the chloroform fraction was reduced to 9.38 ± 0.45 and 4.5 ± 0.31 in the seeds treated with 10 and 20 mg/ml of the fraction (Fig. 3 and 4).

The cytotoxic effects of the extract, the aqueous and chloroform fractions on the tadpoles were also observed to be significantly related to the concentrations with the aqueous fraction exhibiting the highest activity. The lethal effect of these materials was indicated by the complete submergence of the organisms in water. While the extract and the chloroform fraction did not produce any mortality on the tadpoles at concentrations of 10 and 20 µg/ml, the aqueous fraction produced percentage mortality of 33.3 ± 3.3 at 20 µg/ml which eventually increased to 100 % with the concentration of 100 µg/ml in less than 2 h after treatment. However, at a concentration of 200 µg/ml, the extract and the chloroform fraction produce percentage mortalities of 96.67 ± 3.33 and 86.67 ± 6.67 respectively (Fig. 5).

Working on medicinal plants with probable antitumour effects involve series of steps which may be fruitless if the material does not exhibit the claimed effects. In other to avoid wasting materials, certain bench top assay methods have been developed to predict the activities of medicinal plants in inhibiting proliferation of cells. Two of such methods employed in this work were the growth inhibitory of the extract and its organic solvent fractions on the radicles of guinea corn seeds and their lethal effects on the tadpoles of *Raniceps ranninus.*

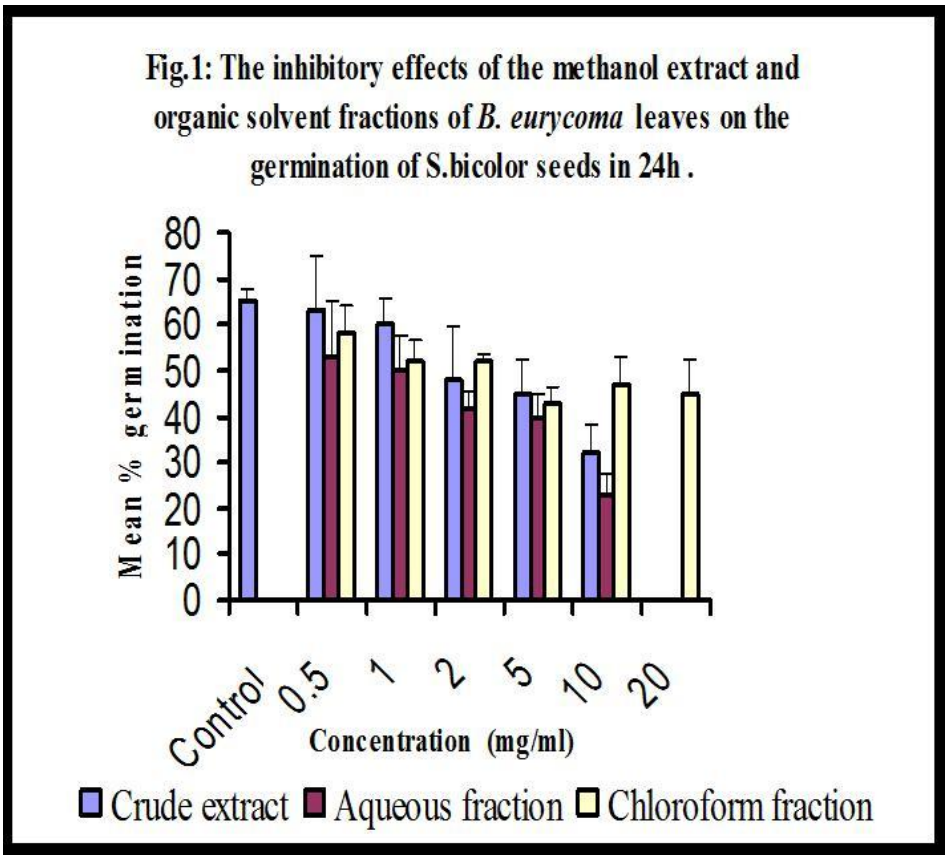


Fig.1: The inhibitory effects of the methanol extract and organic solvent fractions of *B. eurycoma* leaves on the germination of S.bicolor seeds in 24h .

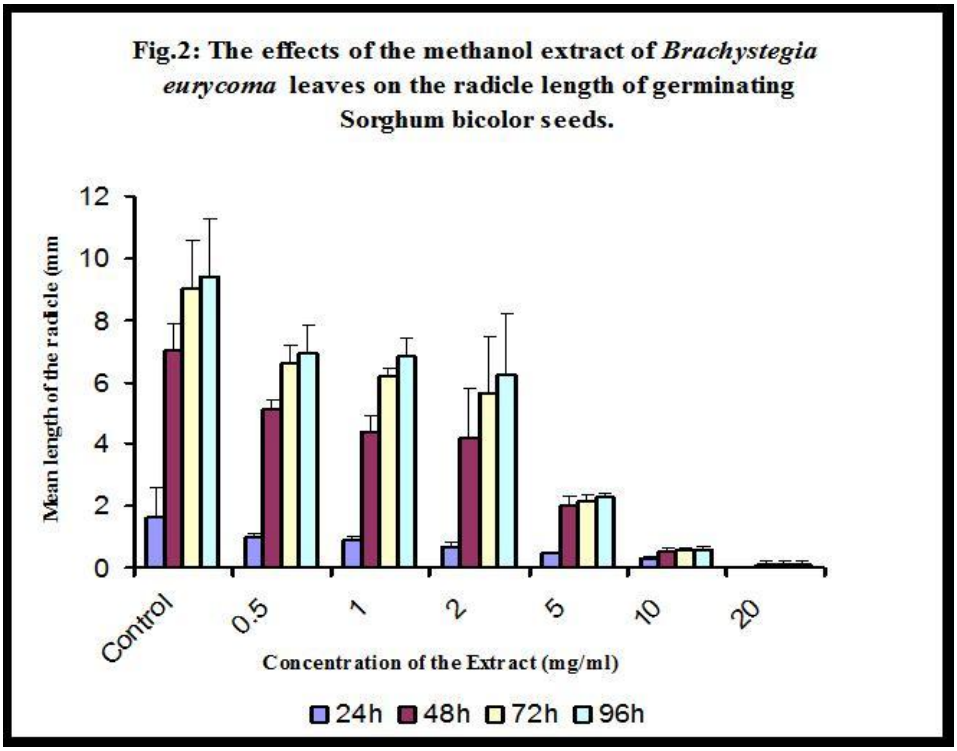


Fig.2: The effects of the methanol extract of *Brachystegia eurycoma* leaves on the radicle length of germinating Sorghum bicolor seeds.

Fig.3:The inhibitory effects of the aqueous fraction of *B. eurycoma* leaves on the radicle length of S.bicolor seeds.

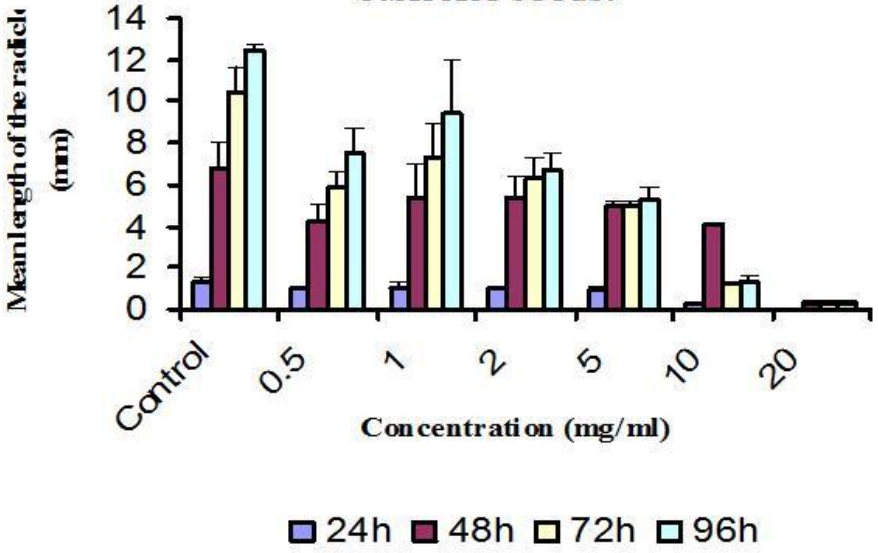
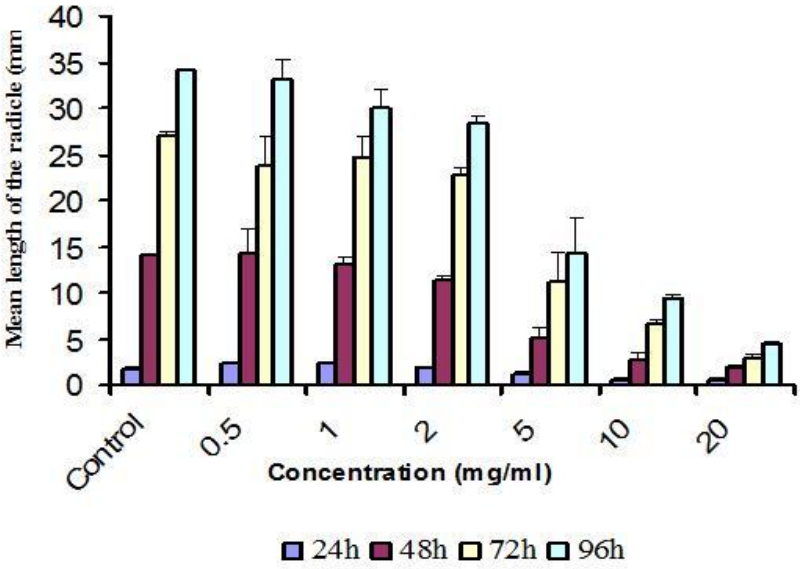
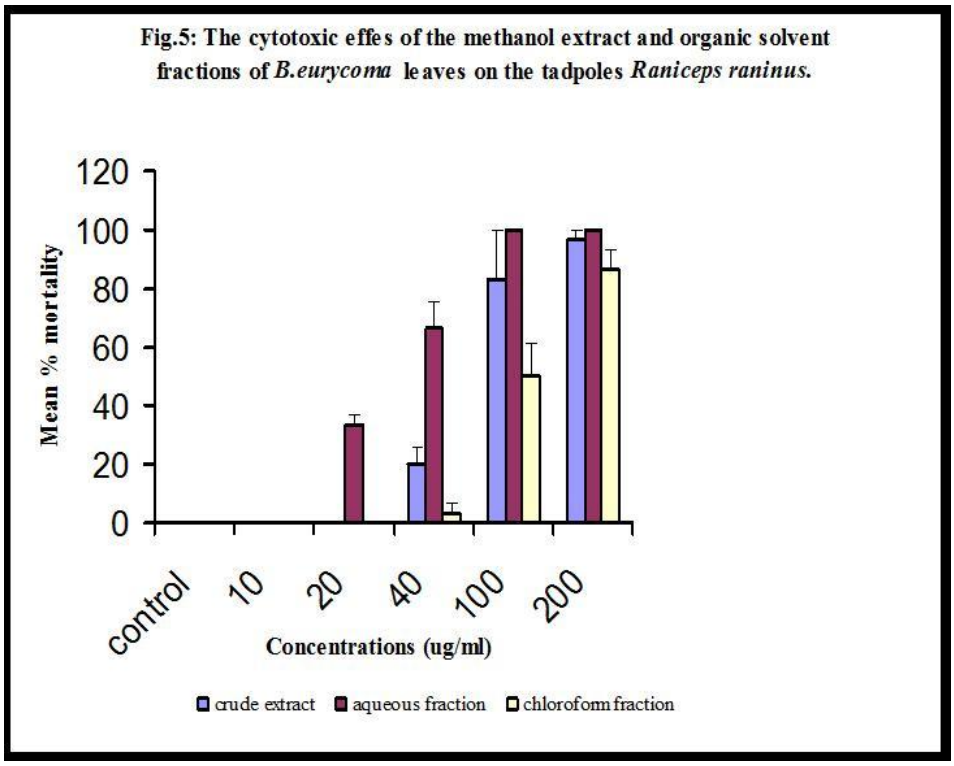


Fig.4: The inhibitory effects of the chloroform fraction of B. eurycoma leaves on the radicle length of S.bicolor seeds.





The activity of medicinal plant extract in inhibiting germination of seeds may be related to induction and promotion of dormancy (Sogbaike *et al.*, 2002). Inhibition of subsequent elongation of radicles has also been related to the probable application of the plant material as herbicidal, allelopathic or antitumor agents (McLaughlin *et al.*, 1991). The plant extract may be exhibiting by interfering with the biochemical system and/ or other growth related systems like DNA division. Furthermore, the ability of plant extracts to impart cytotoxicity on certain organisms like the nauplii of *Artemia salina*, mosquito larvae, and tadpoles has been regarded as a measure of the plant extract to inhibit growth of tumor producing cells **(**McLaughlin *et al.*, 1991; Obuotor and Onajobi, 2000**).** The tadpoles were used in this work due to their availability particularly in the raining season. This of course could be a limiting factor in carrying out this kind of work in the drying season. The fact that the aqueous fraction showed higher activities than the methanol extract on the inhibition of germination, inhibition of proliferation of cells indicated by the length of the radicles and also on imparting toxicity on the tadpoles suggest that the partitioning of the extract resulted in enhanced activities. In addition, it suggests that the constituents of the extract responsible for these activities are polar in nature and it is likely it is same group of constituents. The higher activities of the aqueous fraction over the extract could also be noticed on the IC_{50} of 1.61 mg/ml calculated for the fraction compared to 5 mg/ml observed on the extract. On the toxicity of the materials to the tadpoles, an LC_{50} of 30 µg/ml was observed for the aqueous fraction whereas the extract and the chloroform fraction had LC_{50} of 62.5 and 100 µg/ml respectively.

When compared with previous results, the leaves of *B. eurycoma* can be said to be less toxic to the tadpoles than the leaf extract of *Struchium sparganophora* whose aqueous fraction produced an LC_{50} of 5µg/ml (Ayinde and Agbakwuru, 2010). The results of this work suggest the probable use of this plant in

treating tumour related ailments and its activity is enhanced by further purification.

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