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Cytotoxic and Growth Inhibitory Effects of the Methanol Extract of *Tridax procumbens* Linn (Asteraceae)

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Research into medicinal plants used in treating tumor-related ailments has become imperative due to the emergence of various forms of cancer diseases. *Tridax procumbens* is one of such medicinal plants indicated in traditional herbal medicine as one of the plants used in treating tumor-related ailments. This claim was examined using bench-top assay methods involving the cytotoxicity of the methanol extract of the plant to tadpoles of *Raniceps ranninus* for a period of 24 hr at concentrations between 10-400 µg/ml and the growth inhibitory assay between 1-30 µg/ml. After 24 h, the crude methanol extract and the chloroform fraction produced the highest cytotoxicity of 63.33 ± 1.33 and 100 % respectively at 400 µg/ml. On the growth inhibitory assay, after 96 h, the controls had an average length of 58.12 ± 5.68 mm, whereas the seeds treated with 30mg/ml of the crude extract had an average length of 1.63 ± 0.32 mm, indicating 97.19 % reduction in length. At the same concentration, the chloroform and the aqueous fractions showed 86.58 and 100 % inhibitions. The plant material was observed to contain tannins, saponins and flavonoids, cardiac glycosides and anthraquinone. The result of this work supports the ethno-medicinal use of the plant in preparing recipes for tumor-related ailments.

Keyword: Phytochemical Screening, Crude Extract, Aqueous Fraction, Chloroform Fraction, *Tridax Procumbens*, Growth Inhibition, Cytotoxic Effect.

1. Introduction

The search for new drugs from medicinal plants has led to the isolation and characterization of several bioactive compounds which have been used in the treatment of various ailments^[1]. Bioassay-guided fractionation of medicinal plant extracts for the purpose of isolating bioactive compounds involves a lot of processes and approaches that require time and patients. In random approach to the discovery of new drugs from medicinal plants, plants are collected regardless of their chemical composition or biological activity, but rather on

the availability and abundance of the plant in a particular area^[2].

Tridax procumbens is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. Its widespread distribution and importance as a weed are due to its spreading stems and abundant seed production^[3]. The Yoruba people of Nigeria use the leaf of the plant for treating high blood pressure. In other West Africa sub-region and other tropical countries of the world, traditional medical practitioners and the native people use the leaves of the plant as remedy against conjunctivitis^[4]. In Nigeria, the plant is known with many local names. The Ibo

people call it “mbuli” while in south-western Nigeria it is called Igbalode or Muwagun⁵.

In Nigeria, *Tridax procumbens* is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhoea⁶. *T. procumbens* has been reported to have hypotensive effects⁷ and the whole plant is used against antimicrobial infection⁸ and for healing wounds. The effect of *T. procumbens* on high blood pressure and heart rate on rats and on liver antioxidant defence system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats has been studied^{7,10}. The elemental composition of *T. procumbens* have also been studied¹¹. The aim of this work is to evaluate the cytotoxic and growth inhibitory effects of *T. procumbens* on tadpoles (*Raniceps ranninus*) and guinea corn (*sorghum bicolor*) respectively using established bioassay methods.

2. Materials and Methods

Collection and preparation of the plant materials

The aerial part of *Tridax procumbens* was collected in June 2011 at Igbinedion University Okada and was authenticated at the Forest Research Institute of Nigeria (F.R.I.N.) Ibadan where a Herbarium specimen number FHI109024 was given. The collected plant part was air-dried for 3 days in the laboratory and further dried in the oven at 50 °C. The dried plant was grounded to powder form using a laboratory electric milling machine (Chris Norris, England) and kept in an airtight container until use.

2.1 Extraction of the plant material

1kg of the air-dried and powdered plant material was extracted by maceration for four (4) days using 1.2 L of absolute methanol and the filtrate was concentrated to dryness using a rotary evaporator. The resulting extract was refrigerated maintained at 4 °C until use.

2.2 Preliminary Phytochemical Screening of the Plant Material

The crude extract of *T. procumbens* was subjected to phytochemical screening to detect the presence of secondary metabolites (anthraquinone, tannins,

saponins, flavonoid, steroid, terpenes, alkaloids, and glycosides) using the standard procedures^{12,13,14}.

2.3 Partitioning of the Crude Methanol Extract

Twenty-seven gramme of the crude methanol extract was re-dissolved in methanol-water (1:1) and partitioned exhaustively with chloroform (200 ml ×4) volumes in a separating funnel. The lower chloroform layer was collected followed by the aqueous fraction. This was repeated until a clear lower layer was obtained. The aqueous and the chloroform fractions were concentrated to dryness on a rotary evaporator and their respective yields noted.

2.4 Tadpoles (*Raniceps ranninus*)

Tadpoles of similar sizes were collected from stagnant water in the College of Pharmacy, Igbinedion University Okada, Edo State. They were taken to the Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin where they were identified by Professor M.I. Aisien (an Animal Parasitologist).

2.5 Guinea Corn (*Sorghum bicolor*)

Untreated guinea corn seeds were obtained from a local market in Okada Edo State. A simple viability test was carried out in a 500 ml size beaker containing distilled water and the viable seeds were submerged and nonviable seeds were decanted off. The viable seeds were sterilized with absolute ethanol, rinsed with distilled water and dried before use.

2.6 Determination of the Cytotoxic Effects of the Methanol Extract and Fractions on Tadpoles (*Raniceps ranninus*)

Previously established method was adopted and used to determine the cytotoxic effects of *T. procumbens*. Ten viable tadpoles of similar sizes were collected with the aid of Pasteur pipette (whose narrow end was removed) tube into 50 ml capacity beakers containing 20 ml of the water from the source of the tadpoles which was made up to 35 ml with distilled water. The volume was

made up to 50 ml by making up 0.5, 1, 2, 5, 10, and 20 mg/ml of the leaf extract dissolved in 5 % dimethyl sulphoxide in water to 15 ml thereby making concentrations of 10, 20, 40, 100, 200 and 400 µg/ml respectively. The procedure was repeated for the aqueous and chloroform fractions. For all the extracts the experiment was carried out in triplicates. The controls for each of the experiments were not treated. The mortality rates of the tadpoles were observed for a maximum of 24 h. Tadpole mortality was indicated by the motionless and complete submergence in water.

2.7 Growth inhibitory effects of methanol extract and organic fractions on guinea corn radicle length (*Sorghum bicolor*)

Ten milliliter of 0.5-30 mg/ml of each of the extract and fractions was dissolved in 5 % dimethyl sulphoxide in water and poured into 9 cm wide Petri dishes laid with cotton wool and filter paper (Whatman No 1). Twenty viable seeds of *S. bicolor* were spread on each plate and incubated in the dark. The lengths (mm) of the radicles emerging from the seeds were measured at 24, 72, and 96 hours. The control seeds were treated with 10 ml of 5 % dimethyl sulphoxide in distilled water containing no extracts. The experiments were carried out in triplicates and were repeated for the aqueous and chloroform fractions.

2.8 Statistical Evaluations

Data obtained were analysed using Graphpad Instant R and were expressed as mean ± SEM and one way Analysis of variance (ANOVA). The level of significance was tested and $P < 0.05$ was considered significant. All determination was replicated three times.

3. Results

3.1 Extract Yield and Phytochemical Screening Test

One kilogramme of the plant material yielded 59.55 g (5.955 %) of the extract which further gave a yield of 21.55 g (2.16 %) and 15.23 g (1.52%) of the aqueous and chloroform fractions upon partitioning respectively. Phytochemical

screening of the crude extract revealed the presence of tannins, saponins, flavonoids, cardiac glycosides, terpenoids and steroids. (Table 1).

Table 1: Results of the Phytochemical screening of *Tridax procumbens*

PHYTOCHEMICAL SUBSTANCE	INFERENCE
Alkaloids	-
Saponin	+
Tannins	+
Anthraquinone	+
Flavonoids	+
Cardiac glycosides	+
Steroids	+
Terpenoids	-

+: present, -: absent

3.2 Effects of the extract and fractions on tadpoles.

The crude methanol extract produced percentage mortality of 23.32 ± 0.33 , 30.00 ± 0.58 and 36.67 ± 0.67 and 73.30 ± 1.20 % at concentrations of 20, 40, 100 and 400 µg/ml respectively. Similarly, the chloroform fraction at 200 and 400 µg/ml produced percentage mortality of up to 100 % between 6-13 mins (Figure 1). The aqueous fraction, however gave percentage mortalities of 16.70 ± 0.33 and 36.70 ± 0.13 % at 200 and 400 µg/ml respectively. An LC_{50} of 200, 350.91 and 19.97 µg/ml was observed for the methanol extract, aqueous and chloroform fractions.

3.3 Effect of extract and fractions on Guinea corn radicle growth

After 24 hr., the control seeds showed an average length of 7.68 ± 0.95 mm compared to 2.55 ± 0.72 , 2.10 ± 0.72 , 1.92 ± 0.32 and 1.85 ± 0.29 mm produced by seeds treated with 2, 5, 10 and 20 mg/ml respectively. There was almost complete inhibition of growth in seeds treated with 30 mg/ml of the extract. Similarly, after 96 hr, the control seeds gave an average length of 58.12 ± 5.68 mm as against 8.33 ± 1.29 , 5.93 ± 1.03 and 1.63 ± 0.32 mm produced by seeds treated with 10, 20 and 30 mg/ml respectively.

This implies reduction of 86, 90 and 97 % in the radicle length of seeds with 10, 20 and 30 mg/ml compare to the control seeds (Figure 2). The differences in length of the seed radicle in the treated groups when compared with the untreated control where all significant(P < 0.05).

After 24 hrs. of treatment, the control seeds gave a radicle length of 5.96 ± 0.35 mm compared to 0.95 ±0.11 and 0.31±0.09mm of seeds treated with 5 and 10 mg/ml of the aqueous fraction. At the maximum incubation period, there was

complete inhibition of radicle growth of seeds treated with 20 and 30 mg/ml of the aqueous fraction. (Figure 3).

At 96hr, the chloroform fraction unlike the aqueous fraction which inhibited radicle growth completely, gave an average radicle length of 12.22 ± 0.96 and 7.80 ± 0.08 mm when treated with 20 and 30 mg/ml compare to 49.03 ± 7.55 mm of the control seeds (Figure 4). These variations in length were found to be significant at P < 0.05.

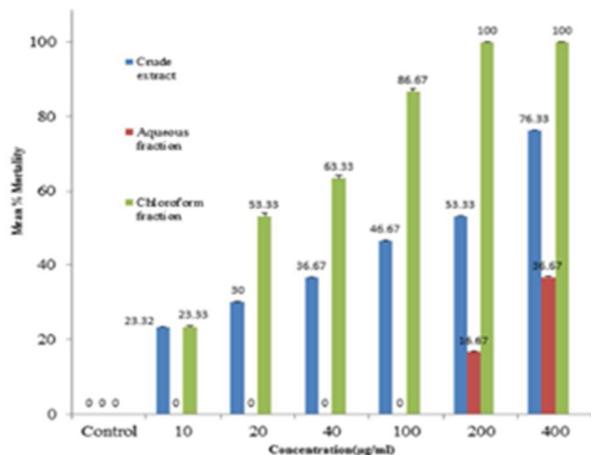


Fig 1: Cytotoxic effects of the methanol extract of *Tridax procumbens* and fractions on tadpoles. Values are Mean ±S.E.M, n= 10
*Significantly different from control. P<0.05

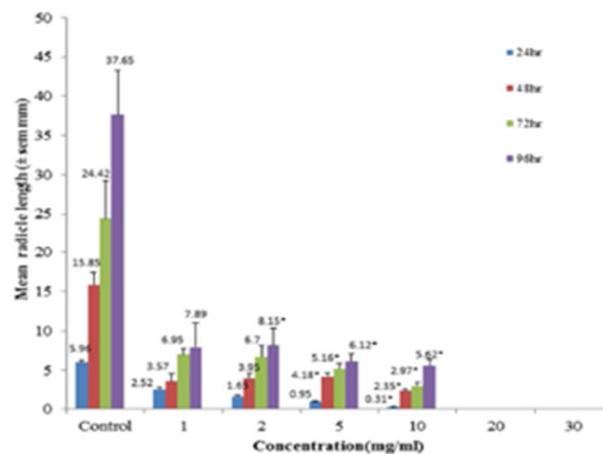


Fig 2 : Growth inhibitory effects of the methanol extract of *T. procumbens* on the radicle length of guinea com. Values are Mean ±S.E.M, n= 20.
*Significantly different from control. P<0.05

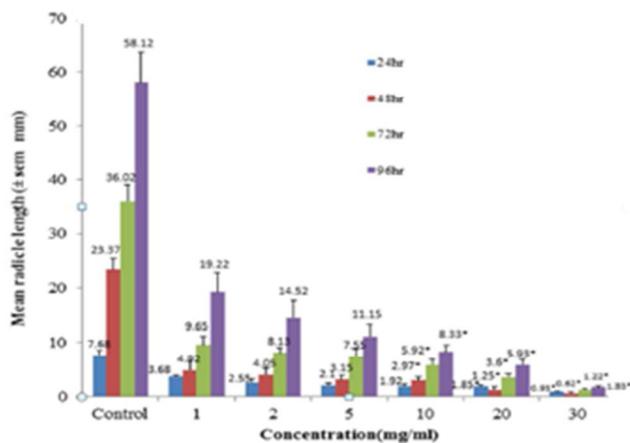


Fig 3: Growth inhibitory effects of the aqueous fraction on the radicle length of guinea com. Values are Mean ±S.E.M, n= 20
*Significantly different from control. P<0.05

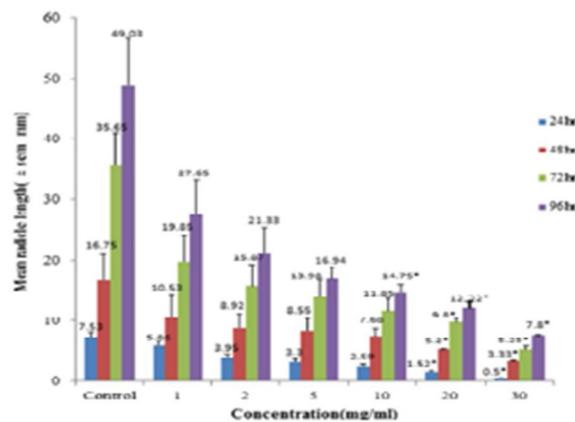


Fig:4: Growth inhibitory effects of the chloroform fraction on the radicle length of guinea com. Values are Mean ±S.E.M, n= 20
* Significantly different from control. P<0.05

4. Discussion

The medicinal properties of plant is due to the presence of mixture of secondary metabolites which are stored in various specialized cells in the various tissues of plants and are usually extracted using various methods. Medicinal plant research currently will continue to be a useful resource in the search for new drugs^[15].

The revival of interest in the use of medicinal plant products for the treatment of various ailments is mainly due to increase awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of currently available synthetic medicines, reported cases of adverse side-effects of modern medicines and perceived gentleness of natural medicines^[16].

Cancer and tumor related ailments which are known to be among the leading causes of death are characterized by uncontrolled cell proliferation in the body, hence the need for research into medicinal plants with probable antiproliferative effects in order to curb the high cost of treating the disease and the life threatening side effects which usually accompany orthodox drugs. Two established simple and rapid bioassay experimental models were adopted to examine the probable cytotoxic and growth inhibitory effects of the of *Tridax procumbens*^[17]. As earlier reported, research work in natural product chemistry must incorporate bioassays^[18]. Extracts must be screened for biological activity, the active extracts selected, fractionations directed with bioassays and the bioactive compounds identified and then exploited.

The choice of guinea corn was informed by the fact that meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions. Although seeds of other plants could have been used, *Sorghum bicolor* was preferred because of their relatively small and their ability to give about 90 % germination rate within 24 hr. Preliminary phytochemical screening of the methanol extract of the plant was carried out to know the class of secondary metabolites it contained and the result showed the presence of saponins, tannins, flavonoids, cardiac glycosides and steroids. Several phenolic compounds have been reportedly linked to have

Cytotoxicity and antiproliferative activities against three melanocytes cell lines^[19].

The methanol extract and fractions were observed to show a significant concentration dependent reduction in radicle length of the seeds and on tadpole mortality rate. For the seeds, it was observed that as the incubation period increases, the length of the radicle continues to show a remarkable reduction when compare with the control. This may be due to the fact that components of the extracts may have interfered with certain biochemical processes directly or indirectly. Partitioning of the crude methanol extract into chloroform and aqueous phase further led to an increase in the cytotoxic and growth inhibitory effects of both fractions.

The aqueous and chloroform fractions like the crude methanol extract inhibited the guinea corn radicle length with increased in concentration, though the aqueous fraction was more effective than the chloroform fraction in this regard. The result of the aqueous and chloroform fraction revealed that the aqueous fraction inhibited the growth of radicle more than the chloroform fraction. Similarly, the tadpoles were more sensitive to the chloroform fraction than the aqueous fraction. This implies that the components responsible for the growth inhibitory and cytotoxic effects are both polar and non-polar respectively.

Comparing the activities of *Tridax procumbens* with other plants like *Struchium sparganophora* it was observed that the latter is more potent than the former. The methanol extract of *S. sparganophora* was reportedly toxic on the tadpoles at 20 µg/ml while chloroform fraction exerted almost complete mortality at a concentration of 80 µg/ml. Furthermore, methanol extract remarkably reduced the growth of the guinea corn radicle at 4mg/ml^[20].

Certain medicinal plants in the Asteraceae (Compositae) family have been reported to possess related activities. The allelopathic effects of *Ambrosia cumanensis* H.B.K. (Compositae) and the leaves and roots of *Piqueria trinervia* (Compositae) have been reported in the literature^[21, 22].

Also, the antiproliferative effects of some Asteraceae (Compositae) species against human cancer cell lines have been observed²³. Furthermore, the cytotoxic effects of *Sonchus oleraceus* and one of its constituents, loliolide, against mice, rat and human cell lines have been established. In the same way, the anticancer effects of the leaves of *Ageratum conyzoides* L. (Compositae) have been established scientifically^[24]. Although this investigation requires further tests using appropriate human cell lines, the results obtained so far have indicated the claimed ethnomedicinal use of these plants in treating tumor-related ailments. The result has shown the cytotoxic and growth inhibitory effects of the *T. procumbens*. However, further research works are needed to fully justify this especially with the use of human cell lines. Also, there is need to establish the constituents responsible for these activities.

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