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Growth Inhibitory and Cytotoxicity Effects of Aqueous Extract of *Musanga cecropioides* R. Br. Ex Tedlie (Urticaceae) Stem Bark

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ABSTRACT

Background and Objective: The treatment of cancer diseases in orthodox practice is plagued with high costs, highly unpleasant side effects and the unavailability of necessary facilities. Therefore, there is a need for sustained search and research into medicinal plants which can be used to complement the efforts towards alleviating the suffering of patients. As a plant member of Urticaceae which contains some plants with reported anti-cancer properties, this study investigated its stem bark for probable anti-cancer properties through anti-proliferative, cytotoxicity and AU565 breast cancer cell line evaluations. **Materials and Methods:** *Musanga cecropioides* stem bark was evaluated for probable anti-cancer properties via bench-top cytotoxicity assay at concentrations of 20-400 µg mL^{-1} and anti-proliferation/growth inhibitory at concentrations of 1-30 mg mL^{-1}. The extract was partitioned into aqueous and chloroform and further tested on the AU565 breast cancer cell line at a concentration of 50 µg mL^{-1}. **Results:** The extract and the fractions produced significant ($p<0.05$) concentration-dependent cytotoxic and growth inhibitory effects on tadpoles and guinea corn radicle. The extract and the aqueous fraction produced 100% mortality in the tadpoles at a concentration of 40 µg mL^{-1} in less than 5 hrs. At 96 hrs incubation period, the radicle length of 47.24±0.48 produced by the control seeds was reduced to 17.56±0.41, 5.73±0.14 and 3.15±0.01 mm in the seeds treated with 1, 5 and 30 mg mL^{-1} of the extract. These implied percentage reductions of 62.8, 87.9 and 93.3%, respectively. The aqueous fraction also showed higher anti-cancer effects than the crude extract and chloroform fraction with 28% cytotoxicity on AU565 cancer cell lines. **Conclusion:** The results of this study have increased the scope of the application of MC stem bark as a crude drug-containing anticancer constituent.

KEYWORDS

Musanga cecropioides, bench-top assay, cytotoxicity, anti-proliferation, growth inhibitory, anticancer, AU565 breast cancer cell line

INTRODUCTION

Many reviews of the status and trends of natural products in drug discovery still confirm the importance of plants as unique therapeutic solutions and as resources for exploration in the search for safer treatments for various ailments[1,2]. Thus, plants remain vita in therapeutics against diseases including cancer. Cancer is characterized by a rapid and uncontrolled formation of abnormal cells that can mass

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together to make growth or tumour, or proliferate throughout the body, initiating abnormal growth at other sites[3]. Plants are known to contain many constituents of various polarities, molecular mass and concentrations[4], of such plant, is *Musanga cecropioides*.

Musanga cecropioides is usually called Umbrella tree or Corkwood, Aga or Agbawo in Yoruba and Oghen by the Edos[5]. Different parts of this plant are employed by traditional healers in the treatment of an array of diseases including stress and depression[6,7], diuretic[8], anti-in ammatory and anti-nociceptive[9,10]. In furtherance of works on the spectrum of ethnomedicinal applications of this plant, this work examined the anti-cancer potential of the aqueous extract of the stem bark using bench-top assay methods including antiproliferative and cytotoxic evaluations using radicle length of *Sorghum bicolor* and tadpoles of *Raniceps ranninus*, respectively as well as breast cancer cell lines.

MATERIALS AND METHODS

Collection and preparation of plant material: The stem bark of *M. cecropioides* was obtained within Ewu Community, Esan-Central LGA, Edo State, Nigeria in May, 2019.

The fresh stem bark of *M. cecropioides* was collected, identified and authenticated at Professor J.C. Okafor Herbarium, Pax Herbal Clinic and Research Laboratories, Ewu, Nigeria and voucher specimen compared with a Herbarium sample deposited in the Department of Pharmacognosy, University of Benin, Benin, Nigeria with specimen number FHT106428.

The plant sample was rinsed and air-dried at room temperature for 14 days and transferred into an oven maintained at 50°C for an additional 4 hrs before pulverization into powder form using an electrical miller (Chris Norris, England). The powdered stem bark (2 kg) of *M. cecropioides* was gradually extracted with water (4 L) using the decoction method for 20 min. The extract obtained was concentrated with an electro-thermal constant water bath to get a brown semisolid paste which was stored in a refrigerator maintained at 4°C for further use.

Partitioning of the crude aqueous extract: About 100 g of the crude aqueous extract was re-dissolved in water and partitioned exhaustively with chloroform (200 mL ×4) using a separating funnel. The lower chloroform layer was collected followed by the aqueous fraction. This was repeated until a transparent lower layer was obtained. The aqueous and the chloroform fractions were concentrated and their respective yields were noted.

Evaluation of growth inhibitory effect of crude aqueous extract and organic solvent fractions on guinea corn (*Sorghum bicolour*) radicle length: Untreated guinea corn seeds were purchased from an area market in Ewu, Edo State. The viable seeds were sterilized with absolute ethanol, rinsed with water and dried before use. Ten milliliters different concentrations (1, 2, 5, 10, 20 and 30 mg mL^{-1}) of the extracts and fractions were poured into 9 cm wide Petri dishes under laid with cotton and filter paper. Twenty viable seeds were spread on each plate and incubated in the dark. The lengths (mm) of the radicles emerging from the seeds were measured at 24, 48, 72 and 96 hrs. The control seeds were treated with 10 mL of water containing no extract. All experiments were triplicated[11].

Evaluation of the cytotoxic effect of the crude extract and the organic solvent fractions on tadpoles (*Raniceps ranninus*): Small and healthy tadpoles of equal sizes were collected from stagnant water within the University of Benin, Ugbowo Campus. Ten small size tadpoles were selected with the help of a broken Pasteur pipette and introduced into beakers containing 15 mL of the water from the source of the tadpoles. This was made up to 49 mL with tap water followed by 1 mL of the various extracts and fractions concentrations of 1, 2, 5, 10 and 20 mg mL^{-1}, the ultimate volume is 50 mL. These correspond to concentrations of 20, 40, 100, 200 and 400 μg mL^{-1} of the extracts in 50 mL, respectively, while the

controls were treated with water. All the experiments were triplicated. The duration and mortality rates were noted and recorded. Tadpole mortality was indicated by the motionless and complete submergence in water[11].

Determination of anticancer activity using AU565 breast cancer cell lines: The stock solutions of plant extract and fractions (20 mg mL^{-1}) were separately prepared in sterile DMSO (100%) and later diluted to 50 µg mL^{-1}. The stock solutions of doxorubicin (1 mM) were prepared in sterile water and kept at -80°C until required. The dilutions were prepared in RPMI-1640 containing gentamycin (50 µg mL^{-1}) on the day of the experiment.

The Au565 cells were cultured in microplates (tissue culture treated, 96 well, flat bottom) to the ultimate volume of 100 uL per well under a specific condition (37°C temperature, 95% O_2 and 5% CO_2) for healthy cell growth. On a subsequent day, when the cells had adhered, the medium was removed from each well. Cells were treated in triplicate with the concentration of test extract and fractions and incubated for 48 hrs. After the period, 200 µL of MTT (0.5 mM) dye was added to all and incubated for 3-4 hrs at 37°C in 5% CO_2. Formazan crystals were dissolved in 100 µL of DMSO and shaken thoroughly for a moment on the shaker. The absorbance of the solution was measured at a wavelength of 570 nm against a background control as blank using a microplate reader[12]. The measured absorbance directly correlates to the number of viable cells. Percentage inhibition of growth of each sample was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{100 - (\text{Mean of OD of test compound} - \text{Mean of OD of blank})}{\text{Mean of OD of DMSO control} - \text{Mean of OD of blank}} \times 100$$

Where:

Blank = Media+dye+cells
DMSO control = Media+cells+DMSO+dye
Test compound = Extract/fraction+cells+media+dye+DMSO

The IC_{50} (concentration at which 50% inhibition of the cells occurred) was calculated by EZ-fit software.

Statistical analysis: All data were expressed as Mean±SEM (Standard Error of the Mean) and n represents the number of treatments used. Where applicable, the data were analyzed using One-way Analysis of Variance (ANOVA), GraphPad Instant version 2.05A software (UK). The level of significance was set to $p<0.05$.

RESULTS

Result of growth inhibitory effect of crude extract and fractions on the guinea corn radicles length: After extraction through decoction, 2 kg of the powdered stem bark of *M. cecropioides* yielded 243.6 g (12.18%) of crude extract. The crude extract (100 g) partitioned between chloroform and methanol yielded 5.5 g (i.e., 5.5%) and 60.3 g (i.e., 60.3%), respectively.

The crude extract was observed to significantly ($p<0.05$) inhibit the growth of the guinea corn radicles in a concentration-dependent manner which was sustained all through the period of the experiment. While the control seeds showed average radicle length of 4.67±0.03 mm in 24 hrs, seeds treated with 1, 2, 5, 10, 20 and 30 mg mL Formazan of the extract showed average lengths of 1.48±0.03, 1.54±0.02, 1.14±0.03, 0.67±0.02, 4.36±0.39 and 1.03±0.01 mm, respectively. For 10 and 30 mg mL^{-1}, these results indicate 86.2 and 78.4% growth inhibitions. Also, in 96 hrs, the control seeds showed average radicle length of 47.24±0.48 mm whereas, seeds treated with 1, 2, 5, 10, 20 and 30 mg mL^{-1} of the extract showed average lengths of 28.57±0.41, 14.33±0.21, 5.73±0.14, 5.73±0.14, 3.2±0.01 and 3.15±0.01 mm, respectively. These again implied 87.9 and 93.3% inhibitions for 10 and 30 mg mL^{-1} in Fig. 1.

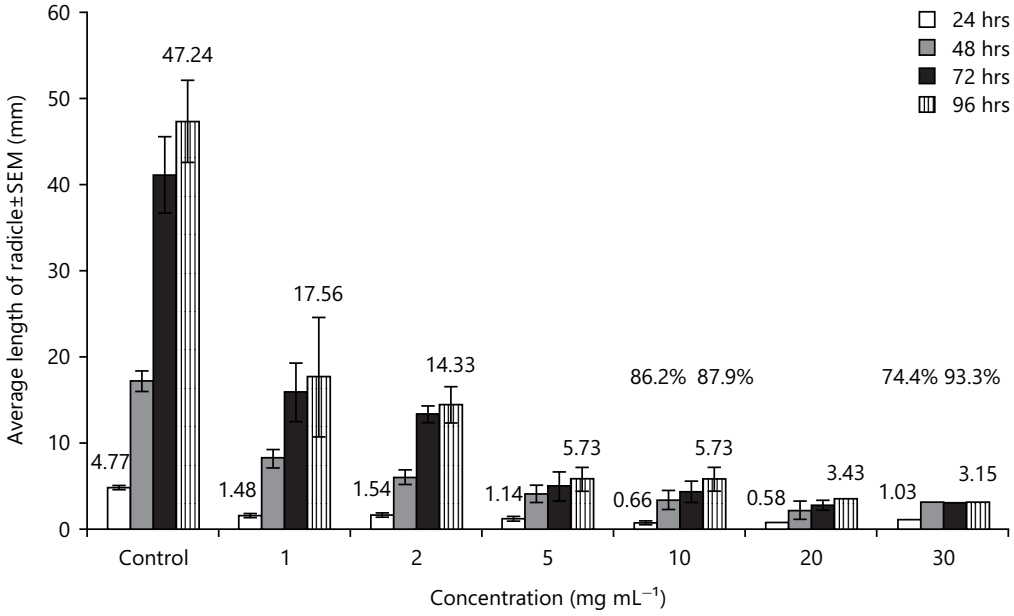

Fig. 1: Growth inhibitory effect of the crude extract on guinea corn radicle length at 24, 48, 72 and 96 hrs
Values are Mean±SEM, n = 20

The extract significantly ($p<0.05$) inhibited the growth of the guinea corn radicles with an increase in concentration.

Both the aqueous and the chloroform fractions also produced concentration-dependent growth inhibitory effects on the radicle lengths of the seeds, although the former was more effective than the latter as enumerated below.

The aqueous fraction was observed to suppress the growth of the radicle length significantly ($p<0.05$) with an increase in concentrations more effectively than the chloroform fraction. At 24 hrs, the control seeds produced an average length of 4.67±0.04 mm compared to 1.48±0.03, 2.05±0.04, 1.22±0.01, 0.79±0.01, 0.51±0.01, 0.37±0.01 and 1.05±0.02 mm produced by seeds treated with 1, 2, 5, 10, 20 and 30 mg mL^{-1} of the aqueous fraction, respectively. There was almost complete inhibition of growth in seeds treated with 30 mg mL^{-1} of the fraction. Similarly, after 96 hrs, the control seeds gave an average length of 47.24±0.48 mm as against 2.8±0.01, 2.67±0.02 and 4.34±0.07 mm produced by seeds treated with 10, 20 and 30 mg mL^{-1}, respectively. For 10 and 30 mg mL^{-1}, the results indicate 89.3 and 90.8% growth inhibitions, respectively in Fig. 2.

A similar trend was seen in the chloroform fraction, while the control seeds showed an average radicle length of 4.67±0.53 in 24 hrs, seeds treated with 20 and 30 mg mL^{-1} of the fraction showed an average radicle length of 2.26±0.46 and 1.6±0.34. Also, at 96 hrs, the control seeds showed an average radicle length of 47.24±2.63 while, seeds treated with 20 and 30 mg mL^{-1} of the fraction showed an average radicle length of 16.97±1.23 and 8.02±0.55, respectively. For 10 and 30 mg mL^{-1}, the results indicate 57.3 and 83% growth inhibitions, respectively in Fig. 3.

Result of cytotoxicity effects of the crude extract, aqueous and chloroform fractions on tadpoles: The crude extract and fractions were observed to produce concentration-dependent cytotoxicity on the tadpoles. While there were no mortalities in the controls, 20 µg mL^{-1} of the crude extract produced 7±0.67% mortality in 24 hrs. The value increased to 100 % mortality at 40 µg mL^{-1}. Similar values were

obtained with the aqueous fraction, however, the chloroform fraction produced 100% mortality at a concentration of 200 µg mL^{-1} in Fig. 4.

Effects of the crude extract and the fractions on AU565 breast cancer cell lines: At the concentration of 50 µg mL^{-1}, the extract was observed not to have an inhibitory effect on the cell line but rather produced a value of -16.89% which indicated a little stimulation of growth above the control. However, the aqueous fraction produced a remarkable growth inhibitory effect as a percentage inhibition of 28.86 was observed in Table 1.

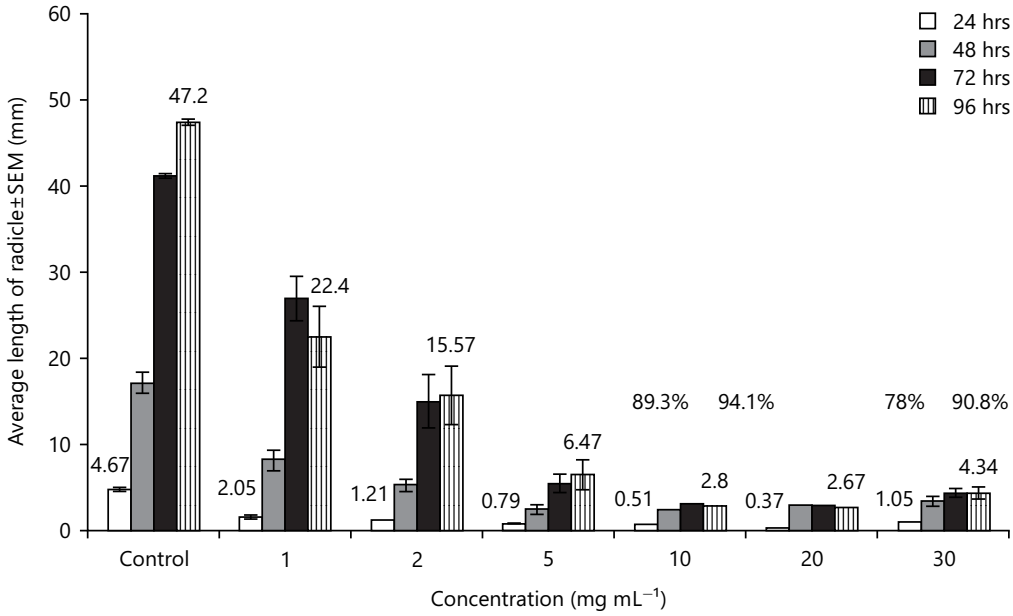

Fig. 2: Growth inhibitory effect of the aqueous fraction on guinea corn radicle length at 24, 48, 72 and 96 hrs

Values are Mean±SEM, n = 20

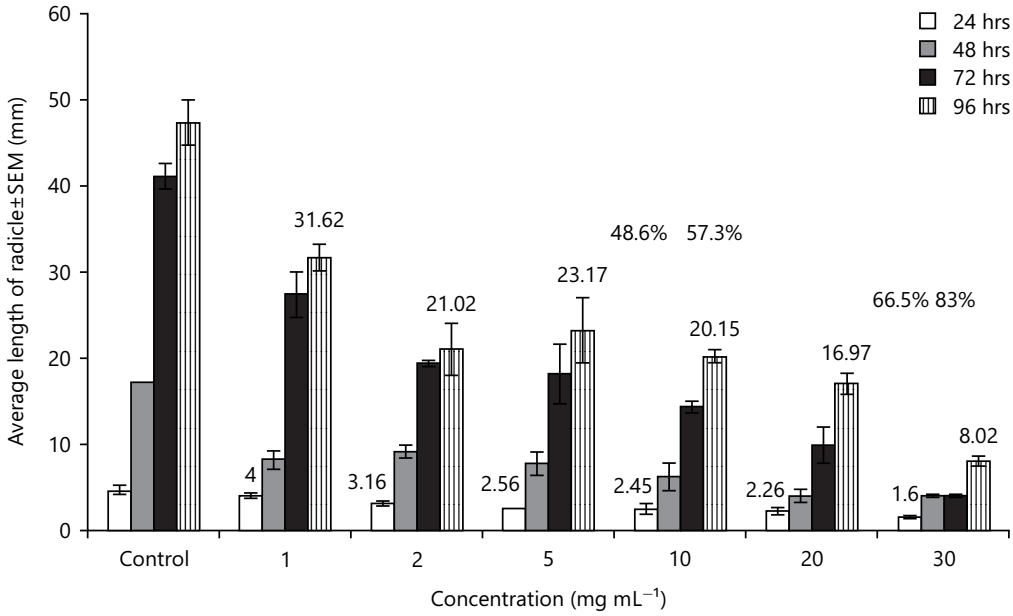

Fig. 3: Growth inhibitory effect of the chloroform fraction on guinea corn radicle length at 24, 48, 72 and 96 hrs

Values are Mean±SEM, n= 20

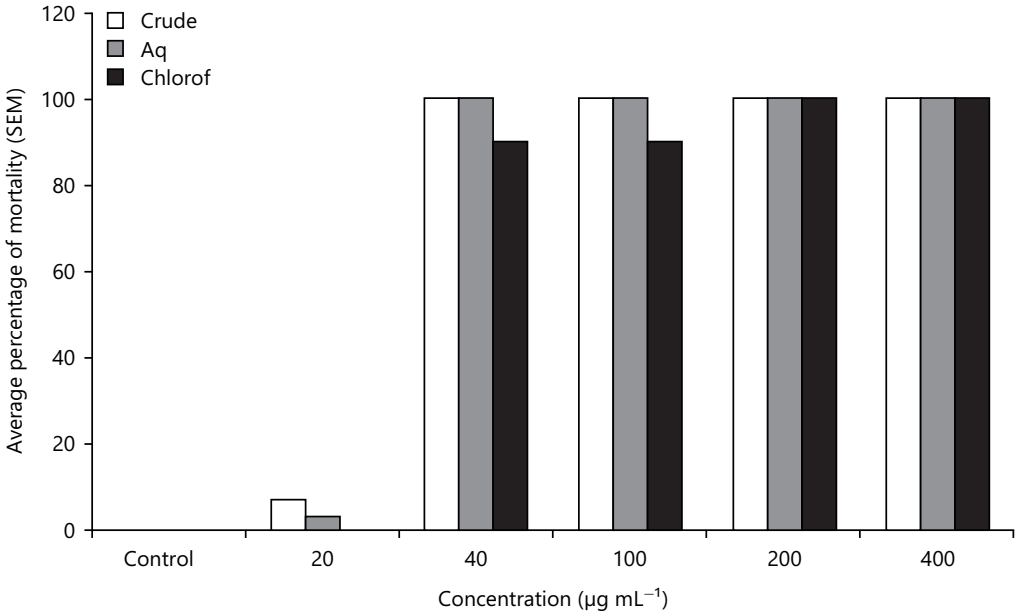

Fig. 4: Cytotoxicity effects of the crude, chloroform and aqueous fractions on tadpoles
Values are Mean±SEM, n= 10

Table 1: Effects of crude extract and fractions of *M. cecropioides* on AU565 breast cancer cell line

Sample (50 µg mL^{-1})	Inhibition (%)
MCC	-16.89
MCcL	Not evaluated
MCA	28.86

MCC: *Musanga cecropioides* crude extract, MccL: *Musanga cecropioides* chloroform extract and MCA: *Musanga cecropioides* aqueous extract

DISCUSSION

From this present study, a yield of 12.18% (243.6 g) from the two kg stem bark of *M. cecropioides* was obtained through the decoction method. Although this method is not suitable for the extraction of thermo-labile constituents of plants, it is known to be faster, cheaper and more convenient compared to other methods like percolation and hot continuous extraction using the Soxhlet apparatus[13]. The decoction method was adopted as it represents the way the crude drug is used in ethnomedicinal practice.

Various bench-top assay methods are used to investigate the potential of plant extracts to impart cytotoxicity on certain zoological organisms just like the tip of *Alum cepa* (onion) root, brine shrimp[14,15], Guinea corn radicle and tadpoles[11] and the results obtained can be used as pointers to the probable anticancer effects of medicinal plants because of their reproducibility and simplicity, other plants have been subjected to such predictive bioassays[16,17].

Partitioning of the crude extracts into chloroform and aqueous phase remarkably improved the cytotoxic and antiproliferative and anticancer effects of the extract against tadpoles, guinea seed radicles and AU656 cancer cell lines. It has been established that fractionation improves activities[18]. In these current evaluations, the aqueous fraction was more effective than the chloroform fraction. For instance, both the crude extract and the aqueous fraction showed 100% mortality at 40 µg mL^{-1} within 4-5 hrs, whereas, the chloroform fraction exhibited 100% mortality on the tadpoles at 200 µg mL^{-1}. Similarly, at a concentration of 10 mg mL^{-1}, the aqueous fraction inhibited seed radicle growth with a reduction of 94.1% in 96 hrs, whereas, at a similar concentration the extract and the chloroform fraction produced 87.9 and 57.3% growth reductions, respectively. Furthermore, while the crude extract did not have any inhibitory effect on the cancer line used, the aqueous fraction at the same concentration of 50 µg mL^{-1} produced 28.86%

growth inhibition. All these imply the probable presence and concentration of the active constituents in the aqueous fraction. Other studies have shown that the predictive methods used in this research translated to anticancer activities[11,19]. The constituents responsible for the observed activities in these studies can be said to be highly polar which explains their solubility in water and this again may have assisted in their rapid absorption by the radicle cells or breast cancer cells where they probably interfered with biochemical or mitotic processes thereby culminating in a drastic reduction in cell multiplication and growth[20].

CONCLUSION

The obtained results have further justified the utilization of bench-top assay methods in anti-cancer screening for medicinal plants. Also, as *M. cecropioides* was not known to have an anti-cancer effect, particularly in breast cancer cell lines, the results obtained can be added to the ethnomedicinal database of the plant as a potential crude drug that can be harnessed and researched further to isolate the major active constituents.

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SIGNIFICANCE STATEMENT

Although *Musanga cecropioides* have been reported to possess some therapeutic effects, there is no such report on its probable potential as an anticancer agent. Thus this study is important to widen the probable medicinal uses of the plant.

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