

CYTOTOXIC AND GROWTH INHIBITORY EFFECTS OF CAMELLIA SINENSIS LINN. (THEACEAE) LEAF METHANOL EXTRACT ON TADPOLES AND GUINEA CORN RADICLE BY BENCH-TOP BIO-ASSAY

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ABSTRACT

Camellia sinensis is indicated in traditional herbal medicine as one of the plant used in treatment of tumor-related ailments in China and India. Cytotoxic and growth inhibitory effects of the methanol extract of leaf was evaluated using the tadpoles of *Raniceps raninnus* and the radicle length of *Sorghum bicolor* seeds respectively. The methanol extract of the leaf produced 70% mortality at a concentration of 200mg/mL and was eventually increased to 90% mortality at the concentration of 400mg/mL over a period of 2 h in the tadpoles. An average growth length of 7.0 ± 2.4 mm was produced by the radicles in the control seeds after 24 h with reduction in values to 5.0 ± 2.0 , 6.1 ± 2.0 , 4.2 ± 0.5 , 3.1 ± 0.5 , 2.3 ± 0.1 and 0.1 ± 0.01 in the seeds treated with 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/mL of the extract concentrations. At 96h, the control seeds recorded a total length of 35.1 ± 12.1 mm in relation to 13.0 ± 5.5 , 11.1 ± 4.3 , 8.5 ± 3.0 , 4.9 ± 1.0 , 4.3 ± 0.1 , 3.7 ± 0.4 and 2.4 ± 1.3 produced by the seeds treated with 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/mL of the leaf extract respectively. The results justified the ethnomedicinal claimed of the plant in the treatment of tumors and cancer.

KEYWORDS: *Camellia sinensis*, cytotoxic, *Raniceps raninnus*, *Sorghum bicolor*.

INTRODUCTION

The interest in investigation the medicinal values of plant in order to explore new way of treating tumor had increased recently. In recent time these has been an increase amount of cancer, research directed towards the investigation of plant derived anticancer compounds. Many of which have been used in traditional herbal treatment for century. Cancer is also one of the major causes of death in developed countries together with cardiac and cardiovascular disease [1]. Cancer can be clinically treated by surgery, radiotherapy and chemotherapy.

After the surgical ablation of progressive cancer, however, metastasized tumor cells continue to progress and this is one of the causes making cancer treatment difficult [2]. Ionizing radiation and most anti-cancer drugs damage DNA suppress DNA replication, killing rapidly growing tumor cells. However, at the same time these adverse effects such as inhibition of bone marrow functioning, nausea, vomiting and alopecia are often witnessed in most cancer therapy [3]. Thus, more effective anticancer drugs with high selectivity against only malignant cells and with the ability to repress tumor metastasis are desired.

As candidate for such drugs cytotoxic antitumor or anticancer natural product have been sought and plant component such as vinca alkaloids, taxoids, etoposide, etc. most of which are from plants are

now used in clinical treatments [4]. *Camellia sinensis* is mainly cultivated in the tropical and subtropical climates, in area with at least 127cm (50 inches) of rainfall per annum. It is commonly called tea and grows in Mambila plateau of Nigeria, as well as in India, China and Kenya [5].

Tea plants prefer a rich and moist growing location in hardiness zones. Many high quantity teas are grown at high elevation up to 1.500 m (4.900 feet); as the plant grows slowly and acquire more flavor. *Camellia sinensis* native to East Asia, the Indian subcontinent and South-east Asia, but it is today cultivated across the world in tropical and subtropical regions including Mambila area of Taraba State, Nigeria.

Camellia sinensis was first used as a food eaten for its medicinal effect most often combined with onion and garlic in Burma. Tea leaves are used for treating headaches, low blood pressure, preventing heart disease, including other sclerosis and heart attack, preventing Parkinson's disease, reducing the risk of stomach and colon cancer, lung, ovarian and breast cancer [6]. The chemical components in tea include alkaloids (thiobromine, caffeine, and theophylline) polyphenols, amino acids, polysaccharides, volatile acid, vitamins, lipids as well as organic elements [7].

This present study was carried out in order to evaluate the cytotoxic and growth inhibitory effects of *Camellia sinensis* leaf methanol extract to ascertain the ethnomedicinal claim of the use of the plant's leaf in treating and prevention of tumors and cancers.

MATERIALS AND METHODS

Collection and identification of plant

Fresh leaves of *Camellia sinensis* were collected in the morning hour from Mambila plateau, Sardauna Local Government Area of Taraba State in April, 2017, and was identified with a voucher specimen number of *THEA001* was deposited at the institute for reference.

Preparation of plant materials

The collected leaves of *Camellia sinensis* were air-dried at room temperature inside the laboratory to avoid decomposition of some chemical substance by sunlight. Dried sample was pounded and ground into powder with the aid an electronic blender. The powdered sample was weighed and kept in an air tight container until required.

Extraction of the plant materials

The powdered leaves weighing 800 g was extracted for 48h in 1000 mL of methanol using Soxhlet apparatus. The extract was concentrated in vacuo in rotary evaporator to obtain a gel-like extract. Final yield of extract was 7.5 % (final weight was 60 g). The extract was stored in desiccators for further use.

Cytotoxic evaluation of *Camellia sinensis* leaf methanol extract

Ten tadpoles were selected into a 250mL capacity beakers, containing 15mL of water from the source of the tadpoles which was made up to 49mL with distilled water. The volume was made up to 50mL with 0.5mL, 1mL, 2mL and 4mL of the extract dissolved in 5% dimethyl sulfoxide (DMSO) in water thereby making the concentration of 10, 20, 30 and 50 mg/mL respectively. The controls for each of the experiment were not treated with the extract and the mortality rate of the tadpoles was observed for a minimum of 24 h.

Determination of growth inhibitory effect of *Camellia sinensis* leaf methanol extract

10mL of 0.5mL, 1mL, 2mL and 4mL of methanol extract was dissolved in 5% DMSO in water, and was poured into 9cm wide petri dishes laid with cotton wool and Whatman No 1 filter paper. Twenty seeds were tested for viability by pre-soaking in 50 mL distilled water and spread on each plate and incubated in a dark cupboard. The lengths (mm) of the radical emerging from the seeds were taken at 24, 72, and 96 h. The control seeds were treated with 10mL of 5% dimethyl sulfoxide in distilled water containing no extracts.

Statistical analysis

The data obtained were expressed as mean \pm SD. One way analyses of variances (ANOVA) was used to test for significance difference. $P > 0.05$ was considered statistically significance.

RESULTS AND DISCUSSION

Production of tumor cells is characterized by an uncontrolled multiplication of the cells. This can be linked to the rapid growth and multiplication exhibited by the meristematic cells of a germinating seed or a growing radicle. The present experiment was conducted to evaluate the cytotoxic and growth inhibitory effect of leaf methanol extract of *C. sinensis* on tadpoles (*Raniceps ranninus*) and guinea corn (*Sorghum bicolor*) radicals respectively.

Result of the current experiment revealed that *C. sinensis* leaf methanol extract has significant growth inhibitory effect on guinea corn radicles. It was observed that the guinea radicles in the control containing 10mL distilled water had the highest length compared to those containing different concentrations after 24, 48, 72 and 96 h respectively. The result also revealed that the length of the guinea corn radicles decreased with increase in concentration of the plant extract (Table 1). Tumor cells are generally known to grow and divide rapidly at an abnormal rate [8]. This is in line with the rapid growth observed in the guinea corn radicles which is due to the presence of the meristematic cells at the root or shoot apex of plants. The guinea corn radicles were observed to grow geometrically within few hours interval as seen in (Table 1).

The ability of the leaf extract of *Camellia sinensis* to inhibit the growth of guinea corn radicles as seen in the result indicated that the plants extract can be used to inhibit the growth of tumor cells which has similar growth trend with the guinea corn radicles. Cytotoxicity study carried out also revealed that the extract has significant cytotoxic effect on tadpoles. In the control experiment containing ten tadpoles (50mL distilled water), there was no mortality recorded. However, in the beaker containing various concentrations of the extract (20, 40, 100, 200 and 400 mg/mL), high mortality rates were recorded after 30 minutes while the beaker containing the highest concentration of the extract (400 mg/mL) recorded 96.7% mortality. The result also revealed that the mortality rate increased with increase in concentration. (Table 2).

Table 1: Growth inhibitory effect of *C. sinensis* leaf extract on *Sorghum bicolor*

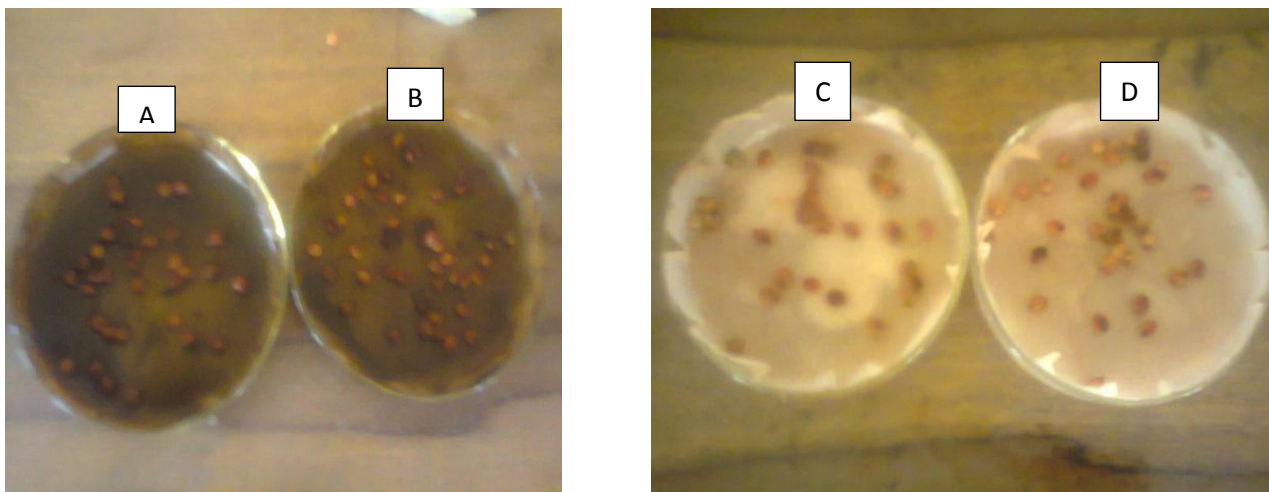
Concentration ($\mu\text{g/mL}$)	Time (hour)			
	24	48	72	96
2.0	5.0 \pm 2.0	5.5 \pm 2.0	6.5 \pm 1.4	13.0 \pm 5.5
2.5	6.1 \pm 2.0	6.5 \pm 2.0	8.3 \pm 3.0	11.1 \pm 4.3
3.0	4.2 \pm 0.5	4.8 \pm 1.0	5.6 \pm 1.3	8.5 \pm 3.0
3.5	3.1 \pm 0.5	3.6 \pm 0.04	4.1 \pm 0.3	4.9 \pm 1.0
4.0	2.3 \pm 0.2	3.0 \pm 0.5	3.3 \pm 0.3	4.3 \pm 0.1
4.5	2.0 \pm 0.1	2.5 \pm 1.0	3.1 \pm 1.0	3.7 \pm 0.4
5.0	0.8 \pm 0.1	0.6 \pm 2.0	2.1 \pm 4.1	2.4 \pm 1.3
Control	7.0 \pm 2.0	47.8 \pm 1.1	29.3 \pm 6.8	35.1 \pm 1.1

n=3, results are mean \pm SD, $p \leq 0.05$ (one-way ANOVA)

Table 2: Cytotoxic effect of *C. sinensis* leaf extract ontadpoles (*Raniceps raninnus*)

Group	Conc (mg/mL)	% mortality rate
Group I	20	10
Group II	40	30
Group III	100	60
Group IV	200	70
Group V	400	90
Group VI	Control	0

$$\% \text{ mortality rate} = \frac{\text{Number of death tadpoles}}{\text{Total number of tadpoles}} \times 100$$

**Plate 1: Growth inhibitory study: A and B; treated plates, C and D; control plates**

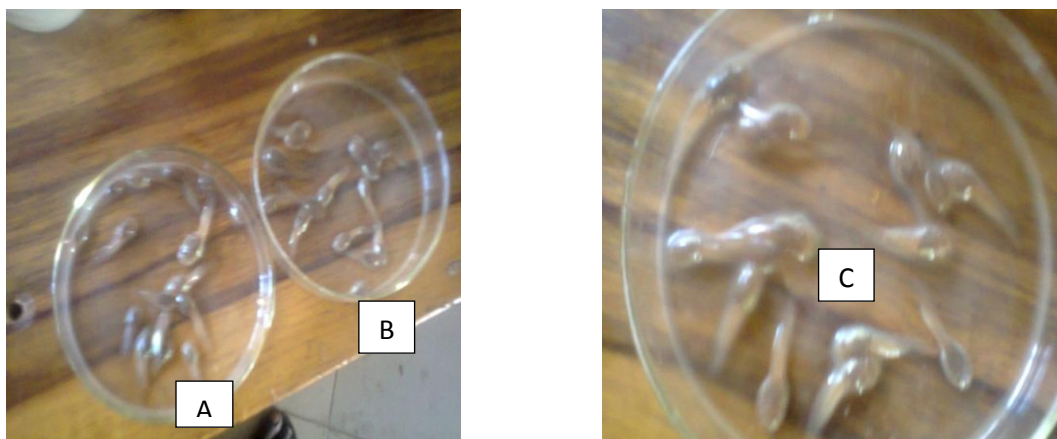


Plate 2: Cytotoxicity study: A and B; tadpoles treated, C; tadpoles in control plate

Research shows that cancer cells divide relentlessly forming solid tumors and are able to spread from one part of the body to another thereby invading other tissues. This can be linked to the fast growing nature of the tadpoles. The ability of the plant extract to kill the tadpoles within 30 minutes indicates that this extract can have a significant cytotoxic effect on cancer cells. These observations are similar to the works by [9], where it was concluded that the plant under observation could be used in treating tumor-related ailments.

Although this investigation requires further tests using appropriate human cell lines, the results obtained so far have indicated the claimed ethno medicinal use of this plant in treating tumor-related ailments. The methanol leaf extract was observed to reduce the rate of germination of the seeds with increasing concentration. It has been reported that at high concentration *C. sinensis* has the ability to inhibit HeLa cell survival compared with vehicle-treated control [10-15]. The length of the seed radicles increased after the incubation period of 24 hours to 96 hours, while those of control seeds increased progressively. It can be inferred that the methanol extract of *Camellia sinensis* was observed to elicit a concentration-dependent reduction in the length of the radicles that emerged from the guinea corn seeds treated with the extract. The cytotoxicity effect was indicated by an initial slowdown in the movement of the tadpoles and subsequent movements, indicated by complete submergence of an organism and turning upside down.

CONCLUSION

From the results obtained in this work, it is concluded that *Camellia sinensis* leaf methanol extract was a potential for causing both anti-proliferative and cytotoxic effects on fast proliferating cells, and hence cancerous cells. This result thus justifies the acclaimed use of the leaf as an anti-tumor and anti-cancer agent in traditional medicine.

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