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# Evaluation of cytotoxic effect of *Tetrapleura tetraptera* (Mimosaceae) pod, root and stem bark extracts on AU565 breast cancer cells

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**Abstract**
*Tetrapleura tetraptera* is a medicinal plant used to treat a variety of diseases, including tumor-related ailments in ethnomedical practice. This study was undertaken to assess its pod, root, and stem bark extracts for cytotoxicity against AU 565 human breast cancer cell line. The plant parts were extracted with methanol and organic solvent partitioning carried out on the pod extract using hexane and chloroform**.** Preliminary screening was conducted on the extracts and fractions with brine shrimp of *Artemia salina* nauplii (10-1000 g/mL) and growth inhibition test with *Sorghum bicolor* seed radicles (5 mg/mL). Antiproliferation effect on AU565 was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 50 µg/mL. The extracts showed moderate cytotoxic activity with *A. salina* nauplii. *T. tetraptera* root extract produced the highest antiproliferative activity, with +99.79 % inhibition on AU 565 cell line. No cytotoxic action was observed with the pod extract on the cell line but its chloroform fraction had high growth inhibitory action on *S. bicolor* radicles and high cytotoxic effect on the cancer cell line, with 81.98 and 82.27% inhibitions realized respectively. The root bark extract and chloroform fraction of the pod extract demonstrated potent cytotoxic activity on the cell line and seem to justify the use of the plant in preparation of recipes for tumor-related ailments.

*Keywords*: AU565; Cytotoxic; MTT; *Tetrapleura tetraptera*

## INTRODUCTION

Plant-based medications are becoming more widely known and used around the world. Food plants utilized for therapeutic purposes abound throughout the African continent. Although there have been advancements in Western medicine, there are still many diseases for which conventional medications have yet to be developed. As a result, there is a pressing need to discover safer medications to treat these disorders. The pharmacological assessment of diverse plants employed in traditional systems of treatment has progressed significantly due to research on medicinal plants. Plants have thus been portrayed as a major source of medicines, not only as isolated active chemicals that are synthesized and distributed in standardized dosage form, but also as crude medications [1].

Cancer is a worldwide problem as it is one of the leading causes of death. It is a condition characterized by uncontrolled cell

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division, which results in aberrant tissue growth [2]. Currently, there are over a hundred different forms of cancer, each with its own etiology and natural history. Breast and cervical cancers are the most common malignant diseases in women among the many cancers [3]. Synthetic medications and chemotherapy used to treat several types of cancer are plagued with prohibitive cost, scarcity, and have life-threatening adverse effects [3]. Therefore, there is a pressing need to assess medicinal plants utilized in ethnomedicine to treat tumor-related diseases in order to exploit their abundance, stated potency, and low or no adverse effects.

*T. tetraptera* Taubert (Mimosaceae) is a deciduous plant which grows on the outskirts of the West and Central African rainforest zone. It thrives in Uganda, Mali, Burkina Faso, Mauritania and countries from Gambia to Nigeria. It grows most luxuriantly in the rain forest, reaching 20-25 m in height, with a girth of about 1.2-3 m [4]. *T. tetraptera* is locally known as *Uyayak* in Ibibio, *Edeminang* in Efik, *Osakirisa* or *Oshosho* in Igbo, *Dawo* in Hausa, *Ighimiakia* in Bini and *Aridan* in Yoruba; all in Nigeria [5]. Because of their pleasant flavor, the fruits are used in Nigeria to make seasoning spices, pomades, and soaps [6,7] while it is used in Ghana as a source of vitamins. It is also commonly used in soups of nursing mothers to prevent postpartum contractions [8]. An infusion of the whole fruit is typically used to treat fevers, constipation, and as an emetic [4] as well as for convulsion, leprosy, inflammation, rheumatic pains, arthritis, diabetes mellitus, hypertension, schistosomiasis, asthma, and microbial infections [9]. The stem bark extract is useful in treating gonorrhea [10]. Concoctions made from the stem bark are also used to cure convulsion [11]. Due to its contents of important phytochemicals, it helps the cardiovascular system and thus it's utilized to prevent and treat heart problems [12].

The presence of caffeic acid in the fruit extract is said to have anticancer properties and to prevent HIV replication [13]. Four saponins isolated from the methanol extract of the fruits using activity-guided fractionation were reported to have substantial molluscicidal activities against schistosomiasis-transmitting snails, *Glabrata biomphelaria* [14]. The cardiovascular and neuromuscular effects of *T. tetraptera* fruit have been reported. It has hypotensive effects in anaesthetized rats, according to pharmacological studies conducted *in vitro* and *in vivo* [15]. Using hydrochloric acid/ethanol-induced gastric ulceration in fasting rat stomachs, the antiulcer effects of the stem bark was demonstrated [16]. The trypanocidal effect of the water extract of *T. tetraptera* against *Trypanosoma brucei* in laboratory rats has been reported [17]. Fresh egg albumin-induced pedal oedema and streptozotocin-induced diabetes mellitus were used to demonstrate the anti-inflammatory and hypoglycaemic effects of *T. tetraptera* fruit aqueous extract in rats [5]. Its anticonvulsant, analgesic and hypothermic activities in mice have also been studied [18]. Sugars, tannins, traces of saponin, and amino acids are known to be present in the fruit and the bark [13].

Based on the ethnomedicinal use of this plant in treating various ailments, this study was carried out to investigate the cytotoxic activity of its pod, root and stem bark extracts on AU 565 breast cancer cell line.

## EXPERIMENTAL METHODS

**Collection and identification of plant materials.** *Tetrapleura tetraptera* pod, stem bark and root bark were collected between September and November 2016 in Ibadan, Nigeria. The identity of the plant material was confirmed by Dr. Shasanya Olufemi, the plant taxonomist at the Forest Research Institute of Nigeria (FRIN) where herbarium specimen number FHI 110614 was assigned.

**Extraction of plant material.** *T. tetraptera* pod, stem, and root bark (1 kg each) were air-

dried for one week before being oven-dried at 50 to 60ºC. With the use of an electric mill, the plant materials were separately reduced to powder. Using a Soxhlet apparatus, the powdered materials were extracted with methanol and concentrated over a water bath maintained at 50°C. The pod extract (60g) was re-dissolved in methanol-water (1:1) and partitioned exhaustively with hexane and chloroform in succession. The fractions were concentrated to dryness and all samples were stored in the refrigerator at 4$^{o}$C until needed.

**Determination of cytotoxic effects on brine shrimp (*Artemia salina*).** About 20 mg of each extract was diluted in 2 mL distilled water, and concentrations of 10, 100, and 1000 µg/mL were obtained from this solution. Using a Pasteur pipette, 10 larvae were introduced into each vial after 48 hours of nauplii hatching and maturation. The vials were filled to a capacity of 5 mL with seawater (38 g/L, pH 7.4) and incubated at 25-27ºC for 24 h under light. Negative and positive controls were provided by vials containing seawater and the reference cytotoxic medication (etoposide) [19].

**Determination of growth inhibitory effects of the pod fractions on *Sorghum bicolor* radicle.** Guinea corn (*S. bicolor*) seeds obtained from Uselu market in Benin City were cleaned with absolute alcohol (95% ethanol), and a simple viability test of the seeds was determined by adding water and immediately decanting thereby eliminating any floating damaged seeds. The ones that stayed submerged in water were chosen and dried before use. The fractions (hexane, chloroform and aqueous) were separately constituted into 5 mg/mL concentrations using 2% Tween 80 in distilled water. Ten (10) mL of each fraction was poured into Petri dishes covered with filter paper (Whatman No 1) underlaid with cotton wool after which twenty (20) viable seeds were scattered on each filter paper and incubated in a dark environment. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72 and 96 h. The control seeds were treated with 10 ml of distilled water containing 2% Tween 80. The experiments were carried out in triplicates for all samples and control [20].

**Determination of cytotoxic activity on AU 565 breast cancer cell line.** The anticancer activity was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. AU 565 breast cancer cell line utilized was obtained from the molecular bank of the International Center for Chemical and Biological Sciences (ICCBS) at the University of Karachi, Pakistan. They were placed in 96-well plates at a density of 10,000 cells/well/100 µL and allowed to incubate for 24 h in complete media at 37 °C and 5% $CO_2$ for the healthy growth of the cells. The next day, when the cells were adhered, the culture media was removed from each well. The cells were treated in triplicates with 50 µg/mL concentration of the extracts and fractions. Doxorubicin (50 µM) was used as the standard. The cells culture was allowed to incubate for 48 hours at 37 °C and in humidified atmosphere of 5% $CO_2$. After the incubation of 48 h, 200 µL of MTT (0.5mM) dye was added in each well and then incubated for another 4 h at 37°C in 5% $CO_2$. The resulting formazan crystals were dissolved in 100 µL of DMSO and absorbance measured at 570 nm. Samples which gave up to 50 % inhibition at 50 µg/mL concentration were considered active and tested at lower concentrations to obtain the $IC_{50}$ which was calculated by EZ-Fit software [21,22].

**Statistical analysis.** All data were presented as mean ± SEM, and significance was determined using One-way Analysis of Variance (ANOVA) statistical test in SPSS version 21. $P<0.05$ was regarded as significant.

## RESULTS

**Cytotoxicity assay on brine shrimp (*Artemia salina*).** The extracts obtained from the pod, root, and stem bark were observed to exhibit

dose-dependent activity. At the highest dose of 1000 µg/mL, the plant extracts produced 26.66, 13.33, and 23.33 % mortalities respectively, indicating that the pod extract showed the highest activity which was far less than the effect of etoposide used as the positive control (Table 1).

**Growth inhibitory effects of the pod fractions on *S. bicolor* seed radicle.** At 24 h, the mean radicle length of the control was 2.82 ± 0.57 mm while those of the hexane, chloroform and aqueous fractions at 5 mg/mL concentration were 1.43 ± 0.17, 1.06 ± 0.25, and 1.36 ± 0.15 mm indicating 49.47, 62.41 and 51.77% inhibitions respectively. At 96 h, the growth of the radicles was greatly retarded by the solvent fractions as 71.74, 81.98 and 64.29 % reduction were produced respectively (Figure 1).

**Cytotoxic effects of the extracts and fractions on AU 565 cell line.** *T. tetraptera* root bark showed the highest cytotoxicity on AU 565 cells at 50 µg/mL concentration with an inhibition of +99.79 % and $IC_{50}$ value of 39.71±2.24 µg/mL. The pod extract produced the least activity of -1.50 % inhibition while +28.50 % inhibition was observed with the stem bark. Solvent partitioning of the pod extract improved its activity remarkably as observed with the chloroform fraction which showed 82.27% inhibition, while the hexane and aqueous fractions gave 34.02 and 16.12% inhibitions respectively (Table 2).

**Table 1:** Cytotoxic effects of the extracts against *Artemia salina*

| Concentration → | 10 µg/mL | 100 µg/mL | 1000 µg/mL | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|
| | Percentage Mortality | | | |
| *T. tetraptera* pod extract | 6.66 ± 0.00 | *23.33 ± 0.00 | *26.66 ± 0.70 | >1000 |
| *T. tetraptera* root extract | 3.33 ± 0.30 | 6.67± 1.90 | 13.33 ± 0.80 | >1000 |
| *T.tetraptera* stem extract | 10 ± 0.47 | 20 ± 0.89 | *23.33 ± 0.00 | >1000 |
| Etoposide | **50.30 ± 0.5 | ***89.10 ± 1.98 | ***100 ± 0.00 | 10.00 |
| Distilled water | 0.00 | 0.00 | 0.00 | - |

Values are expressed as the mean ± SEM of three independent observations. Standard drug; Etoposide $LC_{50}$ = 10 µg/mL. * indicates significance compared to negative control. *P < 0.05, **P < 0.01, ***P < 0.001.

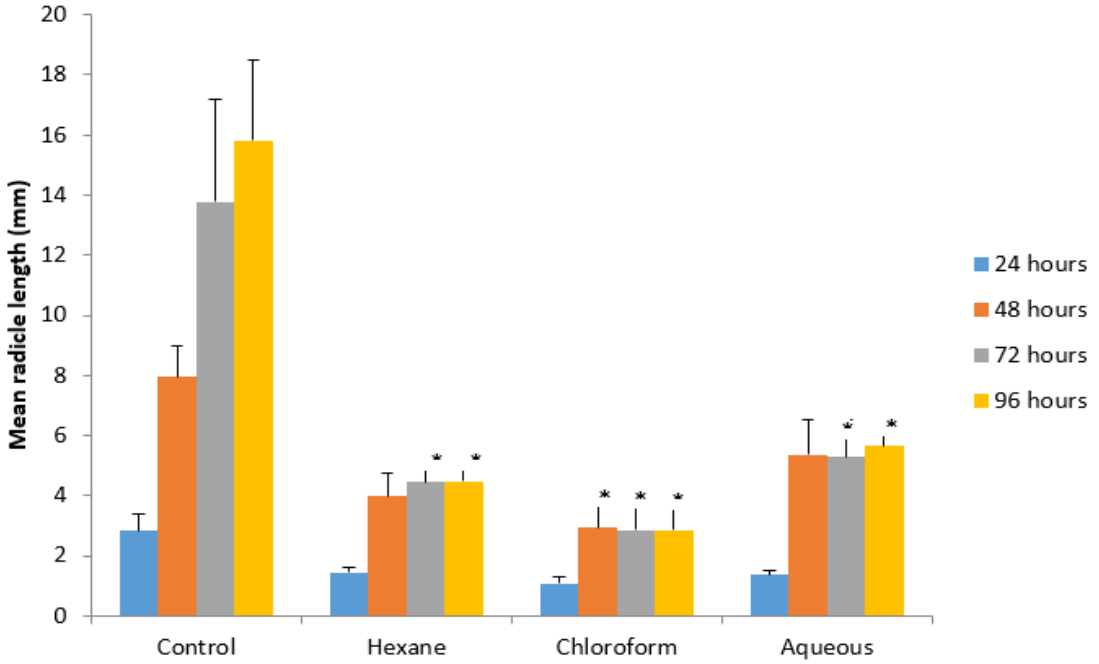


Figure 1: Growth inhibitory effect of the solvent fractions of *T. tetraptera* pod on the radicle length of *S. bicolor* seeds at 5 mg/mL concentration. Values are Mean ± SEM, n = 20. *P < 0.05 compared to control

**Table 2:** Inhibition/stimulation effects of the extracts on AU 565 cell line

| Extracts/Fractions | % Inhibition/Stimulation | $IC_{50}$ |
|---|---|---|
| *T. tetraptera* pod | -1.50 | - |
| *T. tetraptera* root | +99.79 | 39.71±2.24 |
| *T. tetraptera* stem | +28.50 | - |
| Hexane fraction (pod) | +34.02 | - |
| Chloroform fraction (pod) | +82.27 | 9.63 ± 3.61 |
| Aqueous fraction (pod) | +16.12 | - |
| Doxorubicin | +97.89 | 0.085 ± 0.03 |

Each value represents % mean ± SEM of three independent experiments.
$IC_{50}$ = Concentration of extract causing 50% inhibition of cells.

## DISCUSSION

Higher plants have demonstrated their utility as traditional medicines, dietary supplements and spices and they produce a vast variety of secondary metabolites with a wide range of chemical structures [23,24]. Many of the most successful phytomedicines are whole plant extracts, and practitioners think that synergistic interactions between the components of individual or mixes of medicinal plants are critical to their therapeutic efficiency [25].

Antitumor drug research typically entails a series of sophisticated processes that, after a significant investment of time and resources, can yield uninspiring results. Furthermore, due to a lack of research finances, the development and acceptance of simple bench-top tests has become imperative [19]. These bioassays are simple, quick, repeatable, low-cost, and adaptable to a wide range of samples and environments. The brine shrimp lethality test and growth inhibition tests with *Sorghum bicolor* seeds were employed as bench-top assays in this study; cytotoxicity against *Raniceps ranninus* tadpoles and mosquito larvae are other regularly used predictive assays [26].

Lethality of substances to brine shrimp nauplii has been shown to correlate reasonably well with cytotoxicity [27] and also exert a wide range of other pharmacological activities. Plant extracts with $LC_{50}$ values less than 100 µg/mL are deemed bioactive in the brine shrimp lethality test [28]. Higher $LC_{50}$ values, on the other hand, reflect the extract's level of safety. *T. tetraptera* pod, root, and stem bark extracts demonstrated low to moderate cytotoxic activity against *A. salina* nauplii with $LC_{50}$ values greater than 1000 µg/mL, implying the extracts have little toxicity (Table 1).

This plant's growth inhibitory function has previously been described using *S. bicolor* seeds, as well as its cytotoxic effect on *R. ranninus* tadpoles [29]. According to the authors, *S. bicolor* seeds treated with 10 mg/mL root, pod, and stem extracts had radicle length reductions of 78.21, 76.63, and 54.17 % respectively after 96h, which were raised to 87.55, 86.09, and 80.04 % growth reductions with 30 mg/mL concentration. Against *R. ranninus* tadpoles, the root and pod extracts generated 96.67 and 100 % mortality at 20 µg/ml. At this concentration, no mortality was observed with the stem bark extract.

Using MTT assay on AU 565 cells, *T. tetraptera* root extract showed exceptional cytotoxicity, with $IC_{50}$ of 39.71±2.24 µg/mL. Though the pod and stem bark showed reduced inhibitions against the cell line, they have been reported to have promising cytotoxic properties against tumors and other cancer cell lines [30-32]. It's entirely conceivable for a substance to have effect on one type of cancer but not on others.

*T. tetraptera* pod extract reportedly showed antitumor action and improved the life duration of mice by reducing ascites fluid volume and tumor burden [30]. The antiproliferative activity of the pod methanol extract against Jurkat cells and MCF-7 cells

has also been cited [31], furthermore it has been examined against BT 549, BT 20, and T 549 cancer cell lines, where it showed promising cytotoxic activity [32].

The activity of *T. tetraptera* pod extract was considerably boosted by partitioning. The chloroform fraction inhibited radicle growth by 81.98% at 5 mg/mL, according to our findings and $IC_{50}$ of 9.63 ± 3.61 μg/mL against AU 565 cells was also obtained, indicating that a significant portion of the active phytochemicals may be non-polar in nature.

Alkaloids, flavonoids, cardiac glycosides, tannins, steroids, saponins, and anthraquinones have been found in *T. tetraptera* pod and bark extracts [13,33]. Anticancer activities have been discovered in saponins and tannins in particular [34].

**Conclusion.** These findings demonstrate the plants cytotoxic efficacy; here the root bark extract and the chloroform fraction of the pod extract showed the highest cytotoxic effect against AU 565 cancer cell line, albeit more research using other *in vitro* and also *in vivo* assays are needed to confirm these findings.

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