Evaluation of the anti-proliferative and cytostatic effect of *Citrus sinensis* (orange) fruit juice

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**Abstract**

**Aim:** This work has been designed to evaluate the anti-proliferative and cytostatic effects of *Citrus sinensis* (orange) fruit juice on rapidly proliferating cells. **Materials and Methods:** The study was carried out on the seeds of *Sorghum bicolor* for 72 h. The mean radicle length (mm) of the seeds was taken at 48 and 72 h. **Result:** The result showed that when compared with the control, methotrexate, the standard drug showed a significant (*P* < 0.001) anti-proliferative effect throughout the experiment. The inhibition of the radicle growth was more after 72 h (87.42%). At a dose of 5% (v/v), the juice showed a slightly significant (*P* < 0.05) effect after 72 h; however, there was no significant effect at 48 h. The juice at doses of 10% and 20% (v/v) showed a highly significant (*P* < 0.001) anti-proliferative effect throughout the experiment; however, the percentage inhibitions were higher at 72 h. At 72 h, the percentage inhibition for juice at 10% (v/v) was 72.37% and at 20% (v/v) was 91.96%. The concentrations of 40% and 60% (v/v) showed cytostatic effects as no appreciable growth of the radicles of the seeds was observed throughout the experiment. The percentage inhibition for 40% (v/v) was 100% and 99.72% for 48 and 72 h, respectively, while that for the juice concentration of 60% (v/v) was 100% throughout the study. **Conclusion:** The experiment has shown that *C. sinensis* fruit juice has a potential for causing both anti-proliferative and cytostatic effects on fast proliferating cells and hence cancerous cells.

**Key words:** Anti-proliferative effect, *Citrus sinensis*, cytostatic effect, orange juice

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**Introduction**

Cancer is a complex genetic abnormality that has been rated as one of the leading causes of death worldwide presently. The World Health Organization estimated the death caused by this disease in 2004 to be about 13% of the total deaths that year. It has also been predicted that death caused by cancer may escalate by 2030. The causative agents may be found in water, air, food, ultra-violet light and chemicals (including drugs and even household agents).

One of the profound characteristics of cancer is the sustained and uncontrollable proliferation of the cancerous cells. Although some form of success has been achieved in the treatment of this ailment by the current methods available, improvement of these methods/agents and development of better ones are of great importance. Medical chronicles have shown that nature is a reliable source for the development of new drugs. As a matter of fact, about 60% of the currently used anti-cancer agents have their origin from natural sources. Agents capable of inhibiting proliferation are potential anti-cancer agents, as one of the ways the disease progress in the body is by uncontrollable sustained proliferation. Agents capable of causing cytostatic effect may be used for chemo-preventive purposes against cancer. Consumption of antioxidants has been implicated in the prevention of several disorders, including cancer. *Citrus sinensis* (orange) is a commonly consumed fruit in Nigeria. Many people even extract the juice from the fruit and use it for home purposes and even small scale commercial sale. Scientific reports have shown it to be rich in antioxidants and as a natural source of...
ascorbic acid (vitamin c). Therefore, this work has been designed to evaluate the anti-proliferative and cytostatic effects of *C. sinensis* (orange) fruit juice on rapidly proliferating cells.

**Materials and Methods**

**Materials**

Methotrexate injection (Korea United Pharm. Inc., Seoul, South Korea) was purchased from Tarhaf Pharmacy, Jos.

**Plant material**

*Collection and authentication*

The orange fruits were fruits harvested from a local garden, identified and authenticated at the Federal College of Forestry Jos by Mr. Jeff Azila.

*Extraction*

The oranges were washed and the outer fleshy covering was removed using a sterile knife. After that, the juice from the oranges was extracted using a manual juice extractor. The resultant juice obtained was collected in an aseptic manner into a sterile container. It was stored at 4°C till used.

**Experimental plant (Sorghum bicolor)**

The experimental plant, guinea corn (*Sorghum bicolor*), was purchased from the Angwan-rukuba market, Jos. It was subjected to viability test by placing it in a container with water. The floating seeds were disposed, while the submerged ones were dried and cleansed with alcohol for usage.

**Anti-proliferative and cytostatic evaluation**

The modified method of Ayinde et al. was used for this study. Various concentrations (5, 10, 20, 40 and 60% v/v) of the orange juice were prepared. Methotrexate was made to a concentration 50 μg/mL. The Petri dishes were layered with cotton wool and filter paper (Whatman No.1). Twenty (20) seeds of *S. bicolor* were placed in each of the Petri dishes. The control seeds were treated with 10 mL distilled water, while the methotrexate seeds were treated with 10 mL of 50 μg/mL methotrexate. The test seeds were treated with the different preparations of orange juice as the seeds in each specific Petri dish received 10 mL of a particular concentration (i.e. the seeds in a particular Petri dish were treated with 5% v/v concentration, seeds in another Petri dish received 10% v/v, another received 20% v/v, and the next received 40% v/v, while the seeds in the last Petri dish received 60% v/v). The seeds were incubated in a dark room and observed for growth after 24 h, while the mean lengths (mm) of radicle emerging from the seeds were measured after 48 and 72 h. The percentage inhibition was calculated as [(mean radicle length control - mean radicle length treated)/mean radicle length control] × 100. Percentage growth was calculated as 100 - %inhibition.

**Statistical analysis**

The data obtained were expressed as mean ± standard error mean. Two-way analysis of variance and Bonferroni post hoc test were used to test for significance. *P* < 0.05 was considered significant. Graph pad prism (version 5.02) was used for the analysis.

**Results**

The result showed that when compared with the control, methotrexate, the standard drug showed a significant (*P* < 0.001) anti-proliferative effect throughout the experiment. The inhibition of the radicle growth was more after 72 h (87.42%). The juice at a dose of 5% (v/v) showed a slightly significant (*P* < 0.05) effect after 72 h; however, there was no significant effect at 48 h. The juice doses of 10 and 20% (v/v) showed a highly significant (*P* < 0.001) anti-proliferative effect throughout the experiment; however, the percentage inhibitions were higher at 72 h. The percentage inhibition for juice at 10% (v/v) was 72.37% and for 20% (v/v) was 91.96% at 72 h. The concentrations of 40 and 60% (v/v) showed cytostatic effects, as no appreciable growth of the radicles of the seeds was observed throughout the experiment. The percentage inhibitions for 40% (v/v) were 100% and 99.72% for 48 and 72 h, respectively, while that for the juice concentration of 60% (v/v) was 100% throughout the study [Table 1 and Figure 1].

**Discussion**

Divers anti-cancer agents have been developed and are currently in use today. But these have not been able to effectively curtail the ailment although various levels of successes have been achieved. Also, the fact that most of these therapies have the tendency of causing dangerous adverse effects has led to the search for a more effective anti-cancer agent with less side-effects. It has been stated that measures aimed at preventing the disease (i.e. those that cause cytostatic effect to the cancerous cells) are better

| Treatment          | Mean radicle length (mm) | Percentage inhibition | Percentage growth |
|--------------------|--------------------------|-----------------------|-------------------|
|                    | 42 h                     | 72 h                  | 42 h              | 72 h              |
| Control (H2O)      | 16.60±2.4                | 38.55±5.67            | 0.00              | 0.00              | 100 | 100 |
| MTX (50 μg/mL)     | 3.70±0.46                | 4.85±0.58             | 77.71             | 87.42             | 22.29 | 12.58 |
| Cts juice (5% v/v) | 12.70±0.96               | 31.25±3.03            | 23.49             | 18.94             | 76.51 | 81.06 |
| Cts juice (10% v/v)| 4.75±0.49                | 10.65±1.37            | 71.39             | 72.37             | 28.61 | 27.63 |
| Cts juice (20% v/v)| 2.30±0.39                | 3.10±0.47             | 86.15             | 91.96             | 13.85 | 8.04 |
| Cts juice (40% v/v)| 0.00±0.00                | 0.11±0.08             | 100               | 99.72             | 0.00  | 0.28  |
| Cts juice (60% v/v)| 0.00±0.00                | 0.00±0.00             | 100               | 100               | 0.00  | 0.00  |
than treatment procedures as damage cannot be reverted. Therefore, a search for chemo-preventive agents is also a priority. The application of the radicles of fast growing seeds as a parameter for the testing of suspected anti-cancer agents has been previously applied by Ayinde et al.,[11] McLaughlin et al.,[12] Sogbaike et al.[13] and Obuotor and Onajobi.[14] Cancer cells have a characteristic of fast proliferation, and this is also associated with meristematic cells of seeds (such as S. bicolor) when exposed to favorable conditions.[10] These facts give justification to the use of this method for the study.

The result showed that when compared with the control, methotrexate, the standard drug showed a significant (P < 0.001) anti-proliferative effect throughout the experiment. The inhibition of the radicle growth was more after 72 h (87.42%). The juice at a dose of 5% (v/v) showed a slightly significant (P < 0.05) effect after 72 h; however, there was no significant effect at 48 h. The juice doses of 10 and 20% (v/v) showed a highly significant (P < 0.001) anti-proliferative effect throughout the experiment; however, the percentage inhibitions were higher at 72 h. The percentage inhibition of juice for 10% (v/v) was 72.37% and for 20% (v/v) was 91.96% at 72 h. The concentrations of 40 and 60% (v/v) showed cytostatic effects as no appreciable growth of the radicles of the seeds was observed throughout the experiment. The percentage inhibitions for 40% (v/v) were 100 and 99.72% for 48 and 72 h, respectively, while that of the juice concentration of 60% (v/v) was 100% throughout the study.

C. sinensis fruit juice has been reported to contain anti-oxidants. The literature has documented that anti-oxidants are capable of preventing the occurrence of cancer. Therefore, effects of the juice observed in the experiment may be attributed to the presence of anti-oxidants in the juice.

**Conclusions**

The experiment has shown that C. sinensis fruit juice has a potential for causing both anti-proliferative and cytostatic effects on fast proliferating cells and hence cancerous cells. We therefore recommend further work on the same.

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