

Evaluation of the Synergic Antiproliferative and Cytostatic Effects of *Ananas comosus*, *Citrullus lanatus* and *Citrus sinensis* Juice

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ABSTRACT

Background: The World Health Organization (WHO) has named cancer as one of the most significant cause of death presently, with about 6 million reported deaths in 1996, 11.4 million deaths in 2004, 7.6 million deaths in 2008 and 8.2 million deaths in 2012. However, present postulations reveal that there might be an escalation of cancer mortality to about 15 million by 2030, if the progression of the ailment is not checked. **Aim:** The aim of this study is to evaluate the synergic antiproliferative and cytostatic effects of these fruit (*Ananas comosus*, *Citrullus lanatus* and *Citrus sinensis*) juices. **Materials and Method:** The study was performed on rapidly proliferating seeds of *Sorghum bicolor* and the resulting mean radicle lengths (mm) were taken after 24, 48 and 72 hours. **Result:** The seeds treated with methotrexate showed a significant ($P < 0.05$) inhibition of radicle length growth throughout the experiment, compared with the control seeds radicle. The rate of inhibition after 72 hours was 85.39%. The juice doses of 20 and 40% showed a significant ($P < 0.05$) inhibition of seeds radicle length throughout the 72 hours of study. The inhibition rate was however higher after 72 hours with percentage inhibition of 87.19% at 20% juice concentration and 99.50% at 40% concentration. However, juice concentrations of 60 and 80% gave a cytostatic effect (i.e no radicle growth) throughout the study. **Conclusion:** The study has shown that *A. comosus*, *C. lanatus* and *C. sinensis* mixed juice has a potential for eliciting antiproliferative and cytostatic effects on rapidly proliferating cells.

Keywords: *Ananas comosus*, *Citrullus lanatus*, *Citrus sinensis*, antiproliferative effect

INTRODUCTION

Cancer is a genetic ailment which occurs due to accumulated multistep events that have taken place within the culprit cells. This therefore leads to the alteration of the properties and functioning of such cell. ^[1] The World Health Organization (WHO) has named cancer as one of the most significant cause of death presently, with about 6 million reported deaths in 1996, 11.4 million deaths in 2004, 7.6 million deaths in 2008 and 8.2 million deaths in 2012.

However, present postulations reveal that there might be an escalation of cancer mortality to about 15 million by 2030, if the progression of the ailment is not checked. ^[2, 3] The known hallmark of cancer includes rapid proliferation which is uncontrollable, apoptosis evasion, angiogenesis as well as metastasis. ^[4] Divers medical procedures have since been developed by the scientific community to curtail this ailment. Though some form of success have been achieved using these procedures, they

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have however not been able to tame the ailment as reports have shown that occurrence and death caused by cancer is on the rise.^[3] Even the available procedures have significant short-comings like high toxicity, costly and not readily available especially in rural areas. This therefore calls for an urgent development of ideal agents/procedures that will effectively tame this ailment, yet with little or none of the short-comings currently being experienced.^[5] It has however been revealed that prevention of cancer still remain the best solution. Literature has shown that some natural agents are capable of producing anticancer activities. Fruits and vegetables have been reported to be having this activity. However, their nutritional composite have been identified as the possible cause of this activity.^[6] *Ananas comosus* (pineapple) is a member of the family Bromeliaceae. It has a cylindrically shaped fruit having a scaly brown, green or yellow skin, with a yellowish fibrous flesh and a spiny crown at the top. *A. comosus* is used in traditional medicine as an anti-inflammatory agent, gargle for sore throat, antidote for sea sickness and as a diuretic agent.^[7] Its fruit juice is a valuable source of copper, vitamin B₁, vitamin B₂, vitamin B₃, vitamin B₆, vitamin B₁₂, vitamin C, vitamin E and pantothenic acid. *Citrullus lanatus* (water melon) belongs to the family Cucurbitaceae. It is a vine-like plant with the fruit possessing a juicy fleshy mesocarp and endocarp (mostly pink or deep red), and a thick outer exocarp covering (usually greenish with spots of darker green). Its fruit juice contains vitamins (vitamin A, vitamin C, vitamin E, niacine, pantothenic acid, pyridoxine thiamine and folates), electrolytes (sodium and potassium), phyto-nutrients (carotene-alpha, crypto-xanthin-beta, lutein-zeaxanthin, lycopene), minerals (calcium, copper, maganese and zinc), and carbohydrate. *C. lanatus* is employed traditionally in the management and treatment of renal stones, dropsy, diarrhoea, gonorrhoea and prevention of cardiac attack.^[8] *Citrus sinensis* (orange)

belongs to Rutaceae family. Its fruit is fleshy and soft with numerous seeds, and is covered by a thick rugged covering. Literature has unveiled that *C. sinensis* is rich in antioxidants^[9] and is also a natural fountainhead of ascorbic acid (vitamin c). These fruits (*A. comosus*, *C. lanatus* and *C. sinensis*) are usually consumed in Nigeria as dietary component. However, their fruit juices are extracted for both home usage and commercial purposes. But a new trend now is the mixture of different juices extracted from different fruits. This is believed to be having better health benefit. The juice from the individual fruits has been reported to be having antioxidants which are known to elicit anticancer effect. Therefore, the aim of this study is to evaluate the synergic antiproliferative and cytostatic effects of these fruit (*A. comosus*, *C. lanatus* and *C. sinensis*) juices, using bench-top assay method.

MATERIALS AND METHOD

Materials

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

Plant Material

Collection and authentication

The fruits of *A. comosus*, *C. lanatus* and *C. sinensis* were harvested from a local garden, identified and authenticated at the herbarium department, Federal College of Forestry Jos by Mr. Jeff Azila.

Extraction

The fruits (*A. comosus*, *C. lanatus* and *C. sinensis*) were washed and the outer covering was removed using a sterile knife. After that, the juice from the fruits was extracted separately using a manual juice extractor. The resultant juices obtained was collected individually in an aseptic manner into sterile vessels. They were stored at 4 °C till used.

Experimental Plant (*Sorghum bicolor*)

The experimental plant, guinea corn (*Sorghum bicolor*), was obtained from Angwan-rukuba market, Jos. Then, viability screening was carried-out by placing it in a vessel with water. The floating seeds were disposed, while the submerged seeds were dried for usage and cleansed using alcohol.

Antiproliferative Evaluation

The modified Ayinde *et al.*,^[10] bench-top assay method was adopted for this study. Methotrexate was prepared to a concentration 50 µg/mL. 5 mL from each of the juices were intermixed together to get 15 mL mixed juice solution (containing equal volume of *A. comosus*, *C. lanatus* and *C. sinensis* juices). Concentrations of 20, 40, 60 and 80 % v/v were prepared from the resultant mixed juice solution. Six (6) Petri dishes were layered with filter paper (Whatman No. 1) and cotton wool. Twenty (20) seeds of *S. bicolor* each, were placed in each of the Petri dishes. The control seeds received a treatment of 10 mL distilled water. The methotrexate seeds also received a treatment of 10 mL of 50 µg/mL methotrexate.

The test seeds were treated with the different mixed juice preparations. Specific Petri dishes received treatment of 10 mL of a definite concentration. The seeds in a specific Petri dish were treated with 20% v/v juice concentration, seeds in another Petri dish received 40% v/v concentration, a different Petri dish received 60% v/v juice concentration, while the last Petri dish seeds received 80% v/v juice concentration. The different Petri dishes containing the treated seeds were incubated in a dark room. The mean lengths (mm) of radicle emanating from the seeds were measured after 24, 48 and 72 hours. The percentage inhibition was calculated as $[(\text{mean radicle length control} - \text{mean radicle length treated}) / \text{mean radicle length control}] \times 100$.

Statistical Analysis

The data obtained were expressed as mean \pm 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.

RESULT

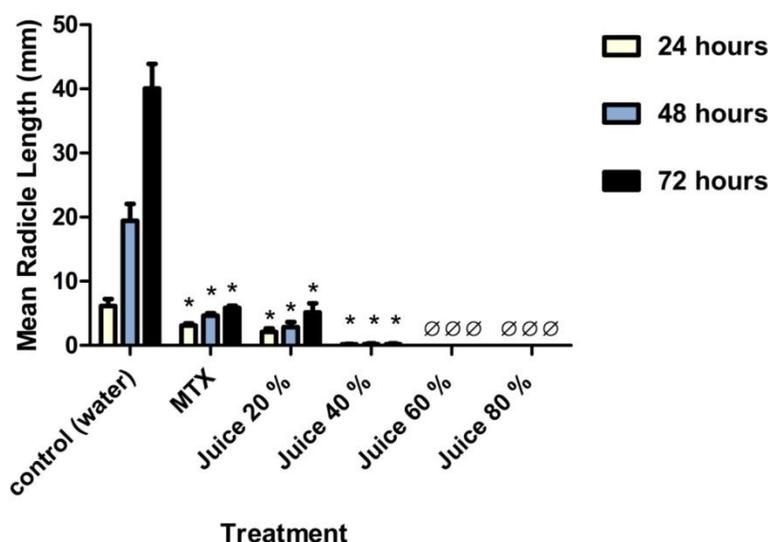


Fig 1: Synergic antiproliferative and cytostatic effects of *Ananas comosus*, *Citrallus lanatus* and *Citrus sinensis* on the growth of guinea corn (*Sorghum bicolor*) radicle. Values are Mean \pm S.E.M, n=20. * = $P < 0.05$, \emptyset = Cytostatic (i.e no growth), MTX =Methotrexate.

Table 1: Synergic inhibitory effect of *Ananas comosus*, *Citrallus lanatus* and *Citrus sinensis* juice on the percentage growth of guinea corn (*Sorghum bicolor*) seeds' radicle

TREATMENT	PERCENTAGE GROWTH %			PERCENTAGE INHIBITION %		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control (Water)	100	100	100	0.00	0.00	0.00
MTX	50.41	23.91	14.61	49.59	76.09	85.39
Juice 20%	34.15	14.62	12.81	65.85	85.38	87.19
Juice 40%	2.44	0.47	0.50	97.56	99.53	99.50
Juice 60%	0.00	0.00	0.00	100	100	100
Juice 80%	0.00	0.00	0.00	100	100	100

MTX = methotrexate, hrs = hours

DISCUSSION

Literature has proven that about 60% of the anti-cancer agents presently used were derived from natural sources including plants. ^[11]

Researches in the field of epidemiology have unveiled the significant involvement of dietary patterns in the prevention of divers ailments including cancer. The regular consumption of various plant products which includes vegetables and fruits have been reported to be involved with the lowering of the risk of cancer occurrence. ^[12, 13] This therefore gives an indication that some fruits may be having significant anticancer activity that can be used in taming cancer. One of the prominent characteristics of cancer cells is high proliferation that is uncontrollable. Hence, the antiproliferative effect of potential anticancer agents on rapidly proliferating cells is now a common parameter used in screening for anticancer agents. The use of radicles of rapidly growing seeds as a parameter for evaluating suspected anti-cancer agents have been previously demonstrated by Sogbaike *et al.*, ^[14] McLaughlin *et al.*, ^[15] Chinedu *et al.*, ^[16] Obuotor and Onajobi ^[17]. Cancerous cells are characteristically known for rapid proliferation, and this is also peculiar with meristematic cells

of seeds (like *S. bicolor*) when exposed to suitable conditions. ^[18] These facts therefore, explain the reason for adopting this method for this study.

The experimental result revealed that the control seeds radicle had a high proliferation rate throughout the period of study. This is therefore a proof of the ability of *S. bicolor* seeds to proliferate rapidly (like cancerous cells) when exposed to suitable conditions. However, the seeds treated with methotrexate showed a significant ($P < 0.05$) inhibition of radicle length growth throughout the experiment, compared with the control seeds radicle. This was due to the inhibitory effect of methotrexate and therefore vindicates its current application as an anticancer agent. The rate of inhibition was 49.59, 76.09 and 85.39% after 24, 48 and 72 hours respectively. The juice doses of 20 and 40% showed a significant ($P < 0.05$) inhibition of seeds radicle length throughout the 72 hours of study. The inhibition rate was however higher after 72 hours with percentage inhibition of 87.19% at 20% juice concentration and 99.50% at 40% concentration. However, juice concentrations of 60 and 80% gave a cytostatic effect (i.e no radicle growth) throughout the

study [fig 1, Table 1]. The inhibitory effect of the fruit juice was dose dependent as higher doses gave better inhibitory effect. All juice doses used for the study gave a better inhibitory effect than that of methotrexate. This therefore shows that the mixed juice formulation of *A. comosus*, *C. lanatus* and *C. sinensis* has a high antiproliferative potential. This effect may be attributed to the presence of antioxidants in all the individual juices making up the mixed juice. Antioxidants as have been previously documented in diverse scientific reports, are

now known for their anticancer characteristics.^[18 - 21] The result obtained from this study correlates with previous research reports on natural products containing antioxidants.

CONCLUSION

The study has shown that *A. comosus*, *C. lanatus* and *C. sinensis* mixed juice has a potential for eliciting antiproliferative and cytostatic effects on rapidly proliferating cells. We therefore propose further studies on it.

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