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Evaluation of the antiproliferative potential of *Cocos nucifera* juice

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ABSTRACT

Introduction: The World Health Organization has predicted that the death caused by cancer may rise to a high rate of about 11.5 million in the year 2030. *Cocos nucifera* juice (Coconut water) is a known refreshing and nutritious beverage which is widely consumed traditionally for its nutritious and medicinal properties. Though assumptions traditionally suggest that it may also have an anticancer property, it is important to scientifically evaluate this property. The aim of this study is to ascertain if *C. nucifera* juice has an antproliferative effect.

Methods: The study was carried-out on rapid proliferating seeds (Sorghum bicolor) and the mean radicle lengths (mm) was taken after 48 and 72 hours.

Results: The control group had an unrestricted progressive proliferation throughout the study. Methotrexate elicited significant ($P<0.001$) antiproliferative effect, with percentage inhibition of 73.9 and 87.6% after 48 and 72 hours. *C. nucifera* juice 20% v/v concentration gave significant ($P<0.01$) inhibitory effect of 49.9% after 72 hours. C. nucifera juice 40% v/v gave a significant ($P<0.01$) effect of 46% and a more significant ($P<0.001$) effect of 49.7% after 48 and 72 hours. *C. nucifera* juice 60% v/v had high significant ($P<0.001$) activity after 48 and 72 hours, giving inhibitory effect of 55.3% and 80%, respectively. *C. nucifera* juice and methotrexate combination had a good synergic effect, eliciting significant ($P<0.001$) inhibitory effect of 73.4% and 80% after 48 and 72 hours, respectively.

Conclusion: The experimental results have unveiled that *C. nucifera* juice has a potential for eliciting antiproliferative effect.

Implication for health policy/practice/research/medical education:
Cocus nucifera juice elicited a significant anti-proliferative activity, hence it may serve as a cost effective tool for cancer prevention.
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Introduction

Studies in the field of epidemiology have unveiled that dietary patterns are significantly associated with the prevention of various chronic diseases such as diabetes, heart disease, Alzheimer's disease and even cancer. The consumption of various natural plant products has however been reported to be highly associated with the reduction of the risk of cancer occurrence (1-3). This has led to the encouragement of consumption of natural plant products as a low cost tool for prevention of such diseases including cancer. Cancer is a major health problem today, as it is one of the leading causes of mortality globally. The World Health Organization has predicted that death caused by cancer may rise to a high rate of about 11.5 million in the year 2030. This by far exceeds the rate of about 7.4 million recorded deaths caused by cancer in 2004 (4). Cancer occurs as a result of events that alters both the properties and functions of the affected cells. However, some of the hallmarks of cancer include uncontrollable and sustained proliferation, evasion of apoptosis, angiogenesis and metastasis (5). The process of the formation of cancerous cells is a complex one, but however when it starts cannot

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be reverted. The progress can only be slowed down or terminated. Therefore, it is now believed that the best treatment for cancer is prevention. *Cocos nucifera* juice (Coconut water) is a known refreshing and nutritious beverage which has been widely consumed traditionally for its nutritious and medicinal properties (6,7). Various medicinal properties of *C. nucifera* juice have been documented (8). Literature has shown that *C. nucifera* juice possesses a potential for boosting the human body antioxidant system (9). Reports have also shown it to be a valuable substitute for oral rehydration and intravenous hydration of patients especially in remote places where they are not readily available (10). It may also offer protection against myocardial infarction and control of hypertension (11,12). It's effectiveness in the treatment of urinary tract infections, mineral poisonings, kidney and urethral stones have been stated (13). It has also been reported to possess significant anti-carcinogenic, antithrombotic and anti-aging properties (14,15). Though assumptions traditionally suggest that it may also have an anticancer property, it is however important to scientifically evaluate this property. The aim of this study is to ascertain if *Cocos nucifera* juice has an antiproliferative effect.

Materials and Methods

Materials

Methotrexate injection (Korea United Pharm. Inc) was purchased from a pharmacy outlet in Jos. Fresh *Cocos nucifera* (coconut) fruit was harvested from a local garden and supplied by a local agricultural personnel in Jos. Authentication was done at the Federal College of Forestry Jos by Mr. J. Azila of the herbarium unit.

Extraction

The *C. nucifera* fruit was de-husked and broken gently, while the liquid endosperm (*Cocos nucifera* juice, i.e coconut water) was collected into a sterile container in an aseptic manner.

Experimental Plant (*Sorghum bicolor*)

The experimental plant *Sorghum bicolor* (guinea corn) was purchased from Angwan-Rukuba market, Jos. A viability test was carried-out on the seeds. This was done by pouring them into a bowl of water and then observed for floating. The floating ones were discarded, while the submerged ones were dried and cleansed with alcohol for usage.

Antiproliferative evaluation

The bench top assay method described by Ayinde et al (16), and modified by Chinedu et al (17), was adopted for this study. A concentration of methotrexate 50 µg/mL was prepared, while different concentrations (20%, 40% and 60% v/v) of the *C. nucifera* juice were also prepared. Six sterile petri-dishes were layered with cotton-wool and filter paper (Whatman No. 1). Twenty seeds of *S. bicolor* were placed into each layered petri-dish. The seeds serving as control were treated with 10 mL of distilled water. The seeds in the methotrexate group were treated with 10 mL of 50 µg/mL methotrexate. The test seeds were treated with different concentrations of *C. nucifera* juice preparation. The first group was treated with 10 mL of 20% v/v concentration, the second group was treated with 10 mL of 40% v/v concentration, and another group was treated with 10 mL of 60% v/v concentration. The last group was treated with 5 ml of 50 µg/mL methotrexate and 5 mL of 50% v/v *C. nucifera* juice. All seeds were incubated in a dark room and observed for radicle growth after 24 hours. The mean length (mm) of the radicles emerging from the seeds in each group was taken after 48 and 72 hours, respectively. The percentage growth was calculated as [mean radicle length treated/mean radicle length control] × 100. Percentage inhibition was calculated as 100 – % growth as well.

Statistical analysis

The data obtained were expressed as mean ± standard error mean (SEM). Two-way analysis of variance (ANOVA) and Bonferroni post hoc test were used to test for significance. GraphPad Prism software (version 5.02) was used for the analysis.

Results

The results showed that the control group had an unrestricted progressive proliferation (growth) throughout the study (Table 1, Figures 1 and 2). The percentage growth was 100% throughout the study, and there was no growth inhibition. All the treated groups showed reduced radicle growth throughout the study (Table 1, Figure 1). Methotrexate had significant ($P<0.001$) effect on radicle proliferation after 48 and 72 hours, with percentage inhibition of 73.9% and 87.6% (Table 1, Figures 1 and 2). *C. nucifera* juice 20% v/v had a significant ($P<0.01$) effect after 72 hours, eliciting an inhibitory effect of 49.9 % (Table 1, Figures 1 and 2). *C. nucifera* juice 40% v/v gave a significant ($P<0.01$) effect of 46% and a more

Table 1. Mean radicle length of Sorghum bicolor seeds treated with *Cocos nucifera* juice

Treatment	Mean Radicle Length (mm)	
	48 h	72 h
Control (water)	18.80 ± 1.79	48.30 ± 4.147
MTX 50 µg/mL	4.90 ± 0.25***	6.00 ± 0.60***
CnJ 20% v/v	15.50 ± 1.55	39.40 ± 4.07**
CnJ 40% v/v	10.15 ± 0.88**	24.30 ± 2.84***
CnJ 60% v/v	8.40 ± 0.81***	12.10 ± 1.02***
CnJ+MTX	5.00 ± 0.61***	7.75 ± 1.463***

MTX = Methotrexate, CnJ = *Cocos nucifera* juice.
** $P<0.01$,*** $P<0.001$, compared to control group.

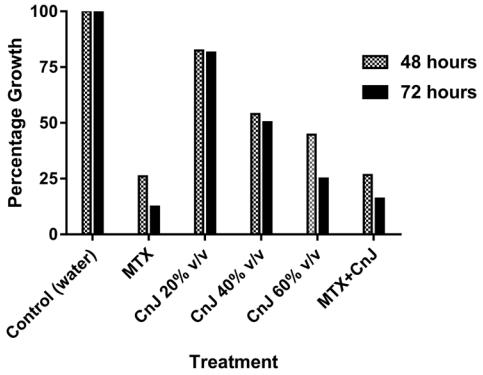

Figure 1. Percentage radicle growth of *Sorghum bicolor* seeds treated with of *Cocos nucifera* juice. MTX = Methotrexate, CnJ = *Cocos nucifera* juice.

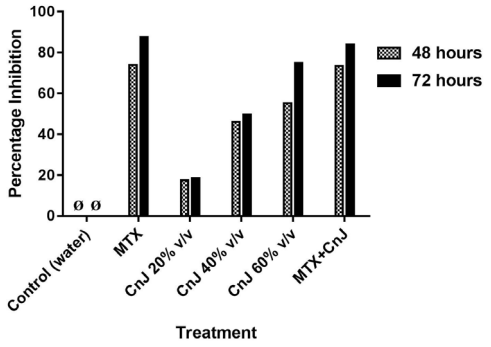

Figure 2. Antiproliferative (growth inhibitory) effect of *Cocos nucifera* juice on *Sorghum bicolor* seeds radicle. MTX = Methotrexate, CnJ = *Cocos nucifera* juice, Ø = no antiproliferative activity

significant ($P<0.001$) effect of 49.7% after 48 and 72 hours, respectively (Table 1, Figures 1 and 2). *C. nucifera* juice 60% v/v had high significant ($P<0.001$) activity after 48 and 72 hours, giving inhibitory effect of 55.3% and 80%, respectively (Table 1, Figures 1 and 2). The combination *C. nucifera* juice and methotrexate had a good synergic effect, eliciting significant ($P<0.001$) inhibitory effect of 73.4% and 80% after 48 and 72 hours, respectively (Table 1, Figures 1 and 2).

Discussion

The search for a better method to curtail the menace of cancer has been a top priority to researchers globally. This is due to the fact that various methods and therapies currently available though have produced some levels of success, have not been able to arrest the condition effectively. As most of them have their short comings like being very expensive, not easily accessible and also having very serious adverse effects. Plant products have now risen to be the main source of drug discovery, as statistics have unveiled that about 3.5-4 million people globally, rely on plant sources for drug (18). History has also unveiled that more than 60% of the currently used anticancer drugs are from natural sources (including plant sources) (19). Since one of the prominent characteristics of cancer is rapid and uncontrollable proliferation, the effect of suspected anticancer agents on proliferation has been used as a criterion for determining their anticancer potential. The use of rapidly proliferating seeds radicle as a parameter for screening of suspected antiproliferative agents have been demonstrated previously by Shogbaike et al (20), Ayinde et al (21), McLauhlin et al (22), David et al (23), and Chinedu et al (24). Cancerous cells are known to show rapid proliferation and this also occurs with meristematic cells of seeds (including *S. bicolor*) under favourable conditions. This explains why this method was adopted for this study.

The results obtained from the study showed that there was a high unhindered growth of the control seeds radicle throughout the study. This reveals that the seeds of *S. bicolor* under favourable conditions proliferate rapidly. The seeds treated with methotrexate had significant ($P<0.001$) decrease in mean radicle growth after 48 and 72 hours compared with control group. This result validates the use of methotrexate as a potent antiproliferative agent. It also reveals that it is capable of inhibiting rapidly proliferating cells of *S. bicolor* just like it does to cancer cells. The inhibitory effect produced was highest after 72 hours, with percentage inhibition of 87.59%. *C. nucifera* juice 20% v/v showed a significant ($P<0.01$) antiproliferative effect after 72 hours, with percentage inhibition of 18.43%. *C. nucifera* juice 40% v/v as well gave a significant ($P<0.01$) antiproliferative effect after 48 hours and even had a higher significant ($P<0.001$) effect after 72 hours, with inhibitory rate of 46.01% and 49.69%, respectively. *C. nucifera* juice 60% v/v had a highly significant ($P<0.01$) antiproliferative effect after 48 hours and even a better significant ($P<0.001$) effect after 72 hours. The percentage inhibition was 55.32% and 74.95% after 48 and 72 hours, respectively. The result showed that *C. nucifera* juice significantly inhibited the proliferation of *S. bicolor* radicle. However, this effect was in a dose dependent order, as higher doses gave a better effect. The effect was also time dependent, as better effects were observed with increase in time (i.e., the effect at 72 hours was better than that at 48 hours). The results from the study therefore give an indication that *C. nucifera* juice, in addition to its numerous medicinal effects already documented (8) may also possess a significant antiproliferative property. The various medicinal effects of *C. nucifera* juice have been attributed its chemical components. Several reports have unveiled the presence of vitamins, fatty acids, amino acids, organic acids, phytohormones, riboflavin, niacin, and vitamin C in *C. nucifera* juice (25-31). Vitamin C, niacin and riboflavin are known antioxidants whose antioxidant properties have been previously documented (24,32). Antioxidants are known to elicit antiproliferative activity and hence are potent anticancer agents (33). The antioxidant property of *C. nucifera* juice has been documented previously by Santos et al (30). Hence, the

antiproliferative activity demonstrated by *C. nucifera* juice in this study may be attributed to the antioxidants (vitamin C, niacin and riboflavin) present in it. The experimental result also showed a very good synergic effect between *C. nucifera* juice and methotrexate, producing a very high significant ($P<0.001$) antiproliferative activity after 48 and 72 hours, with percentage inhibition of 73.40% and 83.95%, respectively. This is also an indication that *C. nucifera* juice may serve as a good combination agent with methotrexate.

Conclusion

The experimental results have unveiled that *C. nucifera* juice has a potential for eliciting antiproliferive effect. This suggests that *C. nucifera* juice may possess potent anticancer activity and may be effective in cancer prevention.

Authors' contributions

All authors contributed significantly towards the success of the work.

Conflict of interests

None.

Ethical considerations

Ethical issues including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy, have been carefully observed by authors.

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