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Original Research Article

Preliminary Growth Inhibitory Studies of the Methanol Extract and Fractions of Leaves of *Artocarpus altilis*.

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Abstract

Purpose: *Artocarpus altilis* is one of the plants used by herbalists in parts of Abraka, Delta State for the management of various forms of cancer. This work is aimed at evaluating this claim using bench-top assay involving anti-proliferation of seed radicles of *Sorghum bicolor*.

Methods: The powdered leaf sample was extracted with 90 % methanol by cold maceration and the extract was evaluated for the presence of phytochemicals. The extract was subjected to vacuum liquid chromatographic fractionation and the fractions were all subjected to biological screening using guinea corn radicle inhibitory assay at concentrations between 1-30 (crude extract) and 1-10 mg/mL (fractions) respectively, over a period of 96 h.

Results: The extract showed the presence of alkaloids, saponins, tannins, cardiac glycosides, flavonoids and anthraquinones. The controls had an average length of 32.45±5.70 mm, whereas the seeds treated with 20 mg/mL and 30 mg/mL of the crude extract had an average length of 2.03±0.87 mm and 0.40±0.28 mm respectively, indicating 93.70 and 98.77 % reduction in length respectively. The bulked vlc fraction A4 was observed to exhibit a higher inhibitory effect over A3 and A10 bulked fractions as it gave an average of 0.65±0.57 at 10 mg/mL after 96 h indicating 99 % reduction in length compared to 46.45 ± 5.32 mm of the control.

Conclusion: This study involving the use of seed radicle assay to evaluate the inhibitory potential of this plant has to an extent validated the traditional use of this plant in the management of various forms of cancer. However, the need to isolate the active ingredients responsible for the observed activity and their corresponding biological activities on human cancer cell lines will form the basis of the next phase of this work

Keywords: *Sorghum bicolor*, phytochemicals, seed radicle, vacuum liquid chromatography

Indexing: Index Copernicus, African Index Medicus

Introduction

All over the world, there is an upsurge of the use of plants not only as food but as medicine. This dependence on plant by man dates back to prehistoric days when there was a need by man to treat various diseases and to restore and fortify his body systems. In striving to maintain a balanced healthy life over a long period of time, man has explored several healthcare methods for the purpose of treating numerous diseases including cancer.

Prior to the emergence of orthodox medicine, treatment of diseases such as cancer and diabetics was totally based on plant-based traditional therapies. This form of medicine involving the use of plant has gained significant ground worldwide as the majority of the world population are still relying on it due to its availability and affordability [1]. In Nigeria, for example, the case is not different as over 60% of the rural populace depends solely on traditional medicine for their health care needs [2]. This can be attributed to lack of adequate health facilities and trained personnel. Although there are some well-known plants with potent activity against some diseases, few have been validated scientifically. Majority of the plants used in this regard possess therapeutic properties owing to the numerous phytochemicals they possess. One such plant is Artocarpus altilis which belongs to the family

Moraceae. Locally known as Pere and Ukwa in Yoruba and Ibo speaking languages of Nigeria [3], *Artocarpus altilis* is commonly referred to as breadfruit due to its similarity to freshly baked bread. Some reported phytochemicals of this plant include geranyl flavonoids, vanillin, norartocarpetin, norartocarpanone, sitosterol, (fruits), artomunoflavonone, artomunoisoxanthone, cyclocomunomethonol (roots), morusin, isocyclomorusin and isocyclomuberrin from the stem [4]. Some significant pharmacological activities of *A. altilis* previously reported includes; antioxidant, anti-inflammatory and antimicrobial activity. Others include the ability to exhibit antidiabetic, hypotensive, anti-tubercular and antiplasmodial activities [5, 6].

Apart from the numerous medicinal uses of this plant such as in the treatment of ear oedema ,ear infections, broken bones, bruises, sprains, abscesses, skin diseases, fungal diseases , diarrhoea stomach-ache and dysentery, cancer, benign prostate hyperplasia and prostate cancer [7, 8],the plant is observed to possess allelopathic properties in the environment where they grow, as they inhibit and sometimes kill another plant around them.

This research is therefore aimed at reporting for the first time the growth inhibitory effects of the extract and vacuum liquid chromatographic fractions of the leaf extract of *Artocarpus altilis*.

Materials and Methods

Materials

Coll1ection and preparation and extraction of the plant material

The leaves of *Artocarpus altilis* were collected in November 2016 at Ebede, Delta state, Nigeria and the identity of the plant was confirmed by Dr. Shasanya Olufemi, a Plant Taxonomist. It was preserved at the Forest Research Institute of Nigeria (FRIN), Ibadan with a herbarium specimen number FHI 109511. The plant material was air dried in the laboratory for 3 days at room temperature, followed by oven drying at 40°C and was subsequently grinded to powder form which was stored in an air tight container.

Extraction of plant materials

One kilogram (1.0kg) of the grinded plant was extracted using cold maceration method with 90 % methanol over a period of 72 h and the extract further dried to paste form using a rotary evaporator maintained at 40°C

Experimental

Preliminary phytochemical screening

Phytochemical evaluation of the extract was done using methods previously described (9, 10, 11).

Evaluation of the anti-proliferative effects of *methanol extract on radicle length* (*Sorghum bicolor*)

Untreated guinea corn seeds (*S. bicolor*) were obtained from Abraka main market and the seeds were tested for viability using the flotation method. Selected seeds (viable) were washed with absolute ethanol for 1 minute and finally rinsed with distilled water and dried before use. About 10 mL of 1-30 mg/mL of each of the extract was dissolved in distilled water and poured into 9 cm wide petri dishes laid with cotton wool and filter paper with 20 seeds of *S. bicolor* were placed on each plate and incubated in the dark for 96 h. The radicle lengths (mm) were measured at 24, 48, 72, and 96 h. The control setup was made of 10 mL of distilled water.

Vacuum liquid chromatography of the extract of Artocarpus altilis.

Forty (40 g) of the crude extract was loaded on a silica gel G (30-70µm) in a Sinta Glass (No.4) attached to a Buchner flask connected to a vacuum pump. The eluting solvents were 300 mL of n-Hexane (100 %), n-hexane and chloroform (1:1 %), n-hexane and chloroform (1:3), chloroform (100 %), chloroform and ethyl acetate (3:1), chloroform-ethyl acetate (1 :1), chloroform and ethyl acetate (1:3), ethyl acetate (100 %), ethyl acetate and methanol (1 :1) and 300 mL methanol (100 %) respectively. Analytical thin layer chromatographic analyses of the vacuum liquid chromatographic fractions were done on a pre-coated aluminum plate of Silica gel GF_{254} using chloroform-methanol (9.4: 0.6). After development, the plates were sprayed with concentrated H_2SO_4 and subsequently heated for 5 min at 110 °C. The colored spots were noted.

The bulked fractions were further screened for growth inhibitory activity using method earlier described above at concentrations between 1-10 mg/mL.

Statistical Analysis

The data's obtained were analyzed using Graph pad Instant R and were expressed as Mean ± SEM and ANOVA and levels of significance measured using Duncan's multiple range test at $P \leq 0.05$, $P<0.01$ and $P \leq 0.001$ respectively

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Results

Yields of the plant extracts

1.0 kg of the powdered leaves of *Artocarpus altilis* yielded 140.80g of the methanol extract corresponding to 14.08 %. Also, 40 g of the extract of the methanol extract of the leaves of *Artocarpus altilis* yielded 4.33 g, 5.35g and 14.96 g of bulked fractions A3, A4 and A10, yielding a corresponding percentage yield of 0.43, 0.54, 1.50 %

Result of preliminary phytochemical screening of the methanol extract of *Artocarpus altilis*

The preliminary phytochemical screening of the crude extract showed the presence of alkaloids, tannins, saponins, anthraquinones, steroids, terpenonids, and cardiac glycosides (**Table 1).**

Table 1: Result of the phytochemical screening of the crude methanol extract of *Artocarpus altilis*

Phyto-constituents	Methanol extract
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Tannins	+
Terpenes	+
Steroids	+
Phlobatannins	-

Keys ; Present: +, Absent: -

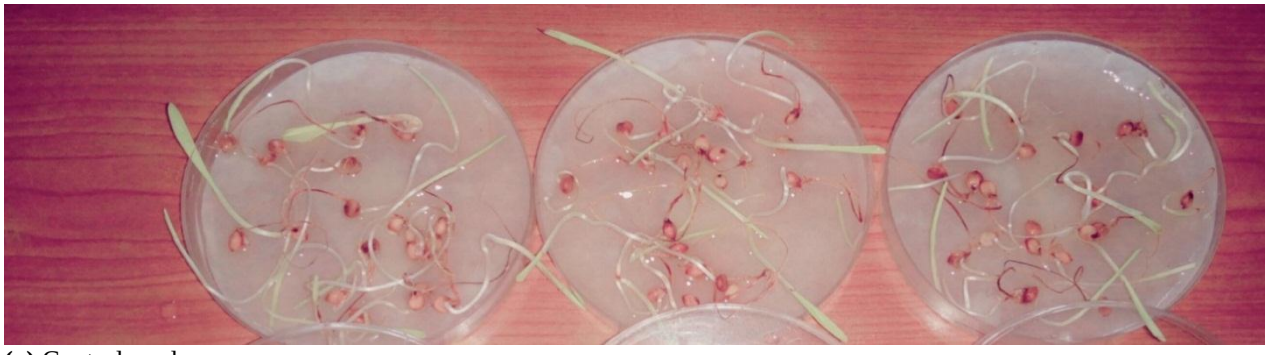
Effect of the crude extract on germinating seed radicles of *Sorghum bicolor*

A concentration-dependent radicle reduction was recorded. The average radicle length of 1.48 ± 0.34 mm produced by the control seeds at 24 h was reduced to 0.53 ± 0.92, 0.18 ± 0.52, 0.16 ± 0.00 and 0.01 ± 0.00 by seeds treated with 5, 10, 20 and 30 mg/mL of the crude extract. This reduction in radicle length was maintained until the end of the experiment (96 h) with the control producing an average length of 32.45 ± 5.70 mm against 12.55 ± 3.29, 5.22 ± 1.83, 2.03 ± 0.87 and 0.40 ± 0.28 produced by seeds treated with 5, 10, 20 and 30 mg/mL of the extract respectively indicating 61, 84, 94 and 99 % reduction in radicle length (Table 2).

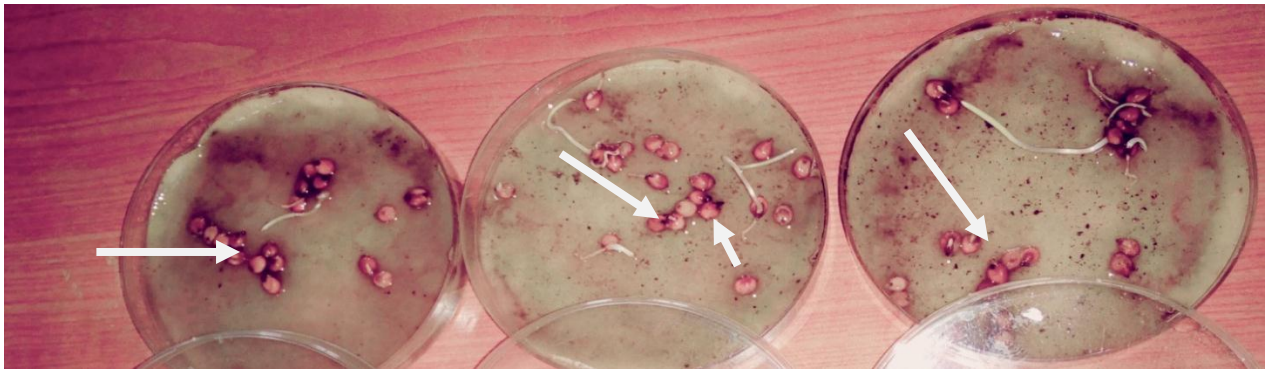
Table 2: Effects of the crude methanol extract of the leaves of *Artocarpus altilis* on radicle length

Concentrations (mg/mL)	Mean radicle length (mm)			
	24 h	48 h	72 h	96 h
Control	1.48 ± 0.34	18.33 ± 3.96	29.68 ± 5.59	32.45 ± 5.70
1	0.88 ± 1.15**	19.27 ± 3.84*	29.48 ± 5.75*	32.43 ± 6.01*
5	0.53 ± 0.92***	8.12 ± 2.22**	11.82 ± 3.06**	12.55 ± 3.29**
10	0.18 ± 0.52***	3.98 ± 1.44***	4.45 ± 1.69***	5.22 ± 1.83***
20	0.16 ± 0.00	1.76 ± 0.65***	1.85 ± 0.85***	2.03 ± 0.87***
30	0.01 ± 0.00***	0.65 ± 0.41***	0.67 ± 0.43***	0.40 ± 0.28***

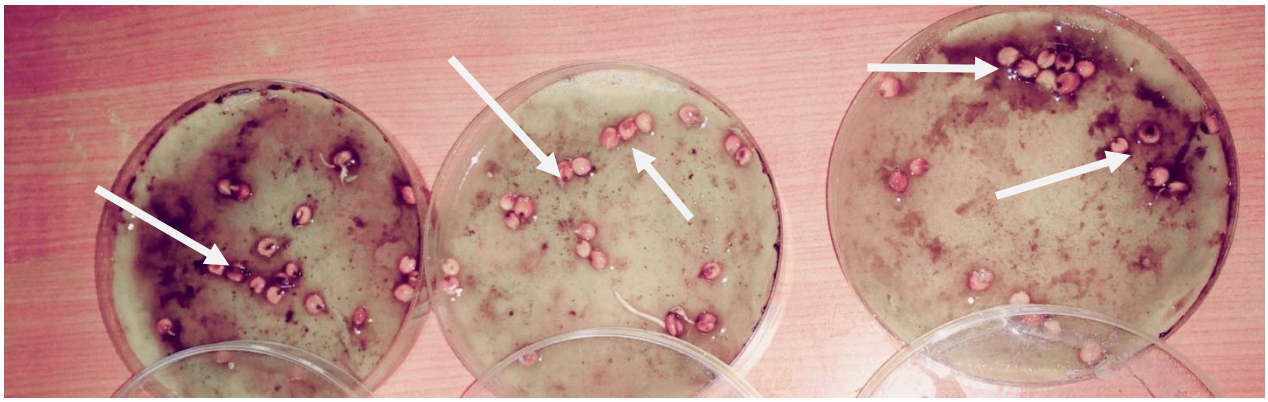
Values are Mean ± SEM, n = 20, *P ≤ 0.05, **p<0.01 ***P ≤ 0.001 significantly different from control



(a) Control seeds



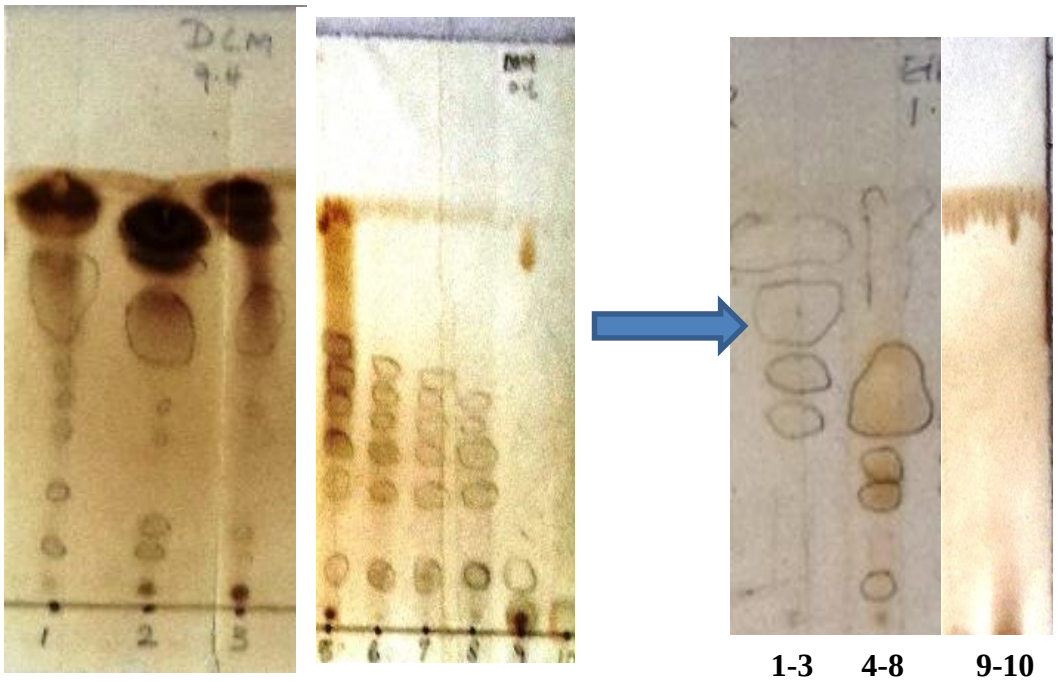
(b) 20 mg/mL



(c) 30 mg/mL

Figure 1: Selected plate showing the effect of the methanol extract of ***Artocarpus altilis*** on the radicle length of *S. bicolor* at 20 and 30 mg/mL. Arrows shows the effects of the extract on seed radicles.

Vacuum liquid fractionation of extract yielded ten (10) fractions which were bulked into three sub-fractions (1-3, 4-8 and 9-10) based on similar TLC (Figure 2). The bulked fractions were coded as A3, A4, and A10



a b

Figure 2: **Chromatogram showing before** (a) **and after bulking** (b) **of the vlc fractions.**

Effect of the vacuum liquid chromatographic fractions on the seed radicles of *Sorghum bicolor*

As earlier observed in the crude extract, the VLC fractions were also observed to exhibit a concentration-dependent results. At the end of 24 h, the control seeds produced a radicle length of 0.98 ± 0.21 mm which was reduced to 0.82 ±0.21 and 0.75± 0.18 mm when treated with 5 and 10 mg/mL of fraction A3. This increase in radicle reduction was similarly observed at 96 h where 31.62±3.83 mm a radicle length produced by the control seeds was reduced to 14.50 ± 2.59 and 12.88 ± 2.58 mm at similar concentrations implying 54 and 59 % radicle length reductions (Table 1)

While the control seeds at 96 h gave 46.45 ± 5.32 mm radicle length, seeds treated with fraction A4 at 5 and 10 mg/mL gave 4.48 ± 1.39 and 0.65 ± 0.57mm radicle lengths respectively which imply 90 and 99 % reduction (Table 3). However, at a similar concentration, bulked fraction A10 produced radicle lengths of 22.33 ± 3.14, 16.50 ± 0.63 mm with an average radicle length of 49.53 ± 7.41mm produced by the control seeds (Table 4).

Table 2: Effects of the VLC bulked fraction **(A3)** of the leaves of *Artocarpus altilis* on radicle length

Concentrations (mg/mL)	Mean radicle length (mm) ± SEM			
	24 h	48 h	72 h	96 h
Control	0.98 ± 0.21	19.08 ± 2.48	29.35 ± 4.51	31.62 ± 3.83
1	0.85 ± 0.16*	12.38 ± 1.95*	19.15 ± 3.06**	22.85 ±3.83**
5	0.82 ± 0.21***	4.72 ± 1.19***	10.87 ± 1.90***	14.50 ± 2.59**
10	0.75± 0.0.18***	4.55 ± 1.03***	8.68 ± 1.70***	12.88 ± 2.58***

Values are Mean ± SEM, n = 20, *P ≤ 0.05, **p<0.01 ***P ≤ 0.001 significantly different from control

Table 3: Growth inhibitory effects of the bulked fraction (A4) of the leaves of *Artocarpus altilis* on radicle length

Concentrations (mg/mL)	Mean radicle length (mm) ± SEM			
	24 h	48 h	72 h	96 h
Control	1.37 ± 0.21	26.72 ± 3.74	45.62 ± 5.71	46.45 ± 5.32
1	0.90 ± 0.18*	11.60 ± 1.74**	20.83 ± 2.73**	21.36 ± 2.34**
5	0.30 ± 0.12**	1.70 ± 0.64***	3.58 ± 1.34***	4.48 ± 1.39***
10	0.02 ± 0.00***	0.12 ± 0.12***	0.30 ± 0.26***	0.65 ± 0.57***

Values are Mean ± SEM, n = 20, *P ≤ 0.05, **p<0.01 ***P ≤ 0.001 significantly different from control

Table 4: Effects of Bulked vlc fraction (A10) of the leaves of *Artocarpus altilis* on Radicle length

Concentrations (mg/mL)	Mean radicle length (mm) ± SEM			
	24 h	48 h	7 2 h	96 h
Control	1.37 ± 0.21	29.38 ± 3.28	45.90 ± 5.73	49.53 ± 7.41
1	1.27 ± 0.18*	21.78 ± 2.88*	35.18 ± 4.76*	34.68 ± 4.60*
5	0.68 ± 0.16***	12.63± 2.07***	19.95 ± 2.85**	22.33 ± 3.14**
10	0.93 ± 0.18***	5.88 ± 0.89***	6.07 ± 0.89***	16.50 ± 0.63***

Values are Mean ± SEM, n = 20, *P ≤ 0.05, **p<0.01 ***P ≤ 0.001 significantly different from control.

Discussion

Research into medicinal plants used in treating tumor-related ailments has become necessary due to the emergence of various forms of cancer diseases and the huge side effects usually encountered by cancer patients when treated with methods such as chemotherapy, radiotherapy and chemically derived drugs [12]. Hence, there is need to look into alternative forms of treatment.

The inhibitory properties exhibited by plant extract could be due to the presence of allelochemicals usually produced by plants as by-products or derivatives of primary metabolites and are translocated to the different morphological parts of the plant where they accumulate [13] and are sometimes released or exude into the environment where they act on neighboring plants. Studies of this kind could serve as an avenue for the discovering of novel secondary metabolites such as phenolics which could serve as potential herbicides and anticancer drugs [14].

Preliminary phytochemical screening of the methanol extract of the plant was carried out to know the class of secondary metabolites it contained and the result showed the presence of saponins, tannins, flavonoids, cardiac glycosides, alkaloids, anthraquinones, and steroids. Several phenolic compounds have been reportedly linked to having cytotoxic and antiproliferative activities against three melanocytes cell lines [15]. This increase in reduction of the seed radicle length could be as a result of the presence of a one or more of these classes of plant secondary metabolites.

Guinea corn seeds (*Sorghum bicolor*), apart from their small sizes, have also been reported to have the ability to proliferate under favorable conditions and germinate within 24 h. These formed the basis of their selection over other seeds. The methanol crude extract and fractions were observed to show a significant concentration-dependent reduction in radicle length of the seeds of *Sorghum bicolor*. For the seeds, it was observed that as the incubation period increases, there was a continuous remarkable reduction in the length of the radicle when compared to the control. This may be due to the fact that components of the extracts/fractions might have interfered with certain biochemical processes directly or indirectly. Also, there was a further continuous decrease in length of the radicle for seeds treated with the fractions with an increase in incubation period when compared to their control.

Fractionation involving the use of vacuum liquid chromatography was observed to increase the activity of the extract. For example, the extract at 10 mg/mL gave 5.22 ± 1.83 mm while seeds treated with faction A4 gave 0.65 ± 0.57 mm after 96 h which implies 84 and 99 % reduction in radicle length. Comparing the inhibitory activities of the different vlc fractions of the leaves extract of *Artocarpus altilis*, it was observed that fraction A4 was more potent than A3 and A10 respectively. For instance, fraction A4 gave an average length of 0.65 ± 0.57 at a concentration of 10 mg/mL

with a percentage reduction of 99 % of the seeds when compared to the control after 96 h while A3 and A10 gave 12.88 ± 2.58 and 16.50 ± 0.63 mm implying 59.27 and 64.48 % reductions respectively. However, the activity of **A4** was observed to be less than that of fraction AQ (4-7), a similar fraction obtained from *Securinga virosa*, which completely inhibited the growth of the radicle of germinating seeds at 10 mg/mL [16].

Conclusion

Based on the results obtained, the leaf of *Artocarpus altilis* may likely have inhibitory effects on proliferative cells, justifying the folkloric use in parts of Abraka, Delta State, in the treatment of various forms of cancer. However, isolation of the active constituents, especially from the active fractions and further biological studies involving the use of human cell lines will form the basis of the next phase of this work.

Conflict of Interest.

There is no conflict of interest linked to this work.

Author Contribution

I declare that this work was carried out by the sole author of this manuscript article. EOI conceived and designed the work.

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