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Anticancer activity of crude extracts from leaves of *Artocarpus heterophyllus*

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Abstract

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. The present study is aimed to study the anticancer activity of crude extracts from leaves of *Artocarpus heterphyllus.* The methanolic crude extract was prepared by maceration extraction method. The extent of cytotoxicity and anti-proliferative effects in the MCF-7 and MDA-MB-231 cells both in the presence and the absence of the leaf extract was determined by the MTT dye reduction assay. The crude extracts of this plants at 62.5 µg/ml did not significantly induce cell death ($p>0.05$, data not shown) comparing the control group. *Artocarpus heterophyllus* plant methanolic crude extract significantly had cytotoxic effects to the MCF-7 and MDA-MB-231 cells ($p<0.05$) comparing to the control group. This plant extract at 1000 µg/ml showed the highest cytotoxic effect (20.81±0.36% of viable cells) with IC_{50} 119 µg/mL. Most of the cells at 1000 µg/ml of extract lost their typical morphology and appeared smaller in size, shrunken, and rounded. This study concludes that, the promising baseline information for the potential uses of the methanol extracts of leaves of *Artocarpus heterophyllus* as an anti-cancer agent.

Keywords: Anticaner, Artocarpus heterophyllus, MTT assay, MCF-7, MDA-MB-231.

INTRODUCTION

A free radical is defined as any molecular species that contains an unpaired electron in the outermost orbital (Halliwell and Gutteridge, 1999). They are highly reactive as they donate or extract an electron hence behave as oxidants or reductants. The most important free radicals produced in the biosystem are oxygen derivatives, particularly superoxide and the hydroxyl radicals which are generated during metabolic activities. If they are imbalanced in the biosystem it leads to oxidative stress resulting in lipid peroxidation, modified DNA bases and protein modification. This result in tissue damage causes many diseases. Hence there is a need in the biosystem to protect from free radical induced damage. (Nordberg and Amer, 2001).

Plants are major resources of antioxidants as they are rich sources of phenolic compounds (Sultana et al., 2007). The antioxidant activity of phenolics is generally combined with hydroxyl groups on their molecules (Perchellet, 1989 and Dragsted, 1998). These natural antioxidants can exert considerable protection, in humans, against aging and cancer caused by free radicals, and can replace synthetic antioxidants such as butylated hydroxyanisole (BHA) and buylated hydroxytoluene (BHT), which are suspected to have toxic and carcinogenic effect on humans (Beier and Oertli, 1983). Employing different long term experimental tumorigenesis protocols, several studies have demonstrated the cancer preventive effects of polyphenolic antioxidants (Hocman, 1989 and Yang, 1997). The cancer chemopreventive effects of polyphenolic antioxidants is specifically important since environmental pollutants, radiation and physical stress exhibit the ability to produce enormous amount of free radicals which cause many diseases, including tumor promotion and cancer (Agarwal, 1993).

Artocarpus heterophyllus (Jack fruit) is a medium size, evergreen tree that typically attains a height of 8-25 m and a stem diameter of 30-80 cm. The canopy shape is usually conical or pyramidal in young trees and becomes spreading and domed in older trees. The tree casts a very dense shade. Heavy side branching usually begins near the ground. All pasrts of the tree exude sticky white latex when injured. This plant is reputed to possess varied medicinal properties. Its use as an

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insecticidal agent has been investigated by several workers. Various phytochemical, pharmacological, antibacterial and antiovulatory studies have already been carried out with the seed extract. Ayurvedic practitioners use stem and leaf extracts as indigenous uterotonic drug. Therefore based on the literature, this plant leaves were selected to study the anticancer properties.

MATERIALS AND METHODS

Plant material

Artocarpus heterophyllus plant leaves were collected from the village Pemberthy, Mandal Hasanparthy of Warangal district which is nearer to Kakatiya University and brought to the laboratory. The plants were identified by a taxonomist from Department of Botany, Kakatiya University, Warangal. The specimens were deposited in Kakatiya University Herbarium.

Preparation of Plant Extract

The leaves were separated from the plant by plucking, washed under running tap water, were shade dried and powdered using an electric blender. These powdered form was stored in airtight containers separately at 4^0C for further use. 500 g of powdered material was weighed accurately, soaked separately in flask containing 1000 ml of 80% methanol for 24 hours at room temperature of 25-30^0C and extracted with maceration method. At the end of the extraction process, the flask containing the solvent extract was removed and excess solvent was evaporated by using rotary evaporator to collect residual crude extract. The residue of the extract was dissolved in methanol and stored in air tight glass vials at 4^0C until further use.

Cell lines and culture conditions

MCF-7 and MDA-MB-231 Breast-adenocarcinoma cell line were established in a permanent culture from human breast carcinoma. These cells were purchased from KIMS (K Institute of medical Sciences), Hyderabad, India. Stock cells are routinely cultured in Dulbecco Modified Eagle's Medium containing 2 mM L-glutamine, 100Units/ml pencillin and 100µg/ml streptomycin at 37°C under an air: CO2 (9:1) or 5% CO2 atmosphere supplemented with 10% foetal bovine serum and the medium was changed for every 48 hours. For the experiments, cancer cells were seeded at a density of 1 x 105 cells/ml in 96 well plates. After 24 hours, medium was changed and the cancer cells were treated with respective drugs. An equivalent amount of DMSO solvent was added to control cells at a final concentration of not more than 0.2%.

Cytotoxicity by MTT assay

The extent of cytotoxicity and anti-proliferative effects in the MCF-7 and MDA-MB-231 cells both in the presence and the absence of the leaf extract was determined by the MTT dye reduction assay as described by Igarashi and Miyazawa (2001). After incubation of cultured MCF-7 cells and MDA-MB-231 cells for 24 h, the cells were washed with fresh medium and were treated with different concentrations of selected medicinal plant methanolic crude extracts as well as standard drug. After 24 hours, 100µl of MTT (0.5 mg/mL final concentration) solution was added to each well and incubated for another 4 h at 37^0C under 5% CO_2. The MTT solution was removed and DMSO (100 µL) was added to each well. The amount of formazan salt was determined by measuring the OD at 570 nm wavelength by microplate reader (Robonix, Japan). The optical density of the control cells were fixed to be 100% viable and the per cent viability of the cells in the other treatment groups were calculated using the formula,

$$\% \text{ of Cell Viability} = \frac{\text{Absorbance of Control - Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

A graph was plotted with concentration (µg/ml) on X axis and % cell viability on y axis and IC50 values were calculated, which represents the concentration of the compound that caused 50% cell viability.

Morphological analysis

Morphological changes in MCF-7 and MDA-MB-231 cells exposed to increasing concentrations (250 µg/ml, 500 µg/ml and 1000 µg/ml) of methanolic crude extract of leaves of *Artocarpus heterophyllus* were taken. For this experiments were randomly divided into control group and extract treated groups. Control group were untreated cells and extract treated group were cells treated with 250 µg/ml, 500 µg/ml and 1000 µg/ml of each plant methanolic leaf extract. Exponentially growing MCF-7 and MDA-MB-231 cells were seeded onto a 96-well plate at a density of 1 x 10^5/ ml cells/well. Methanolic extract of each plant was added to each well separately. After incubation at 37°C for 24 h, morphological cell changes were observed under an inverted microscope at 20x.

Statistical analysis

The results were presented as mean ± SD (Standard Deviation) of sextuplet wells. The parameters were analyzed with one-way analysis of variance (One-way ANOVA) followed by Sidak's multiple comparisons test. Significant levels were considered at $p < 0.05$ and highly significant level at $p < 0.01$ comparing with control group.

RESULTS AND DISCUSSION

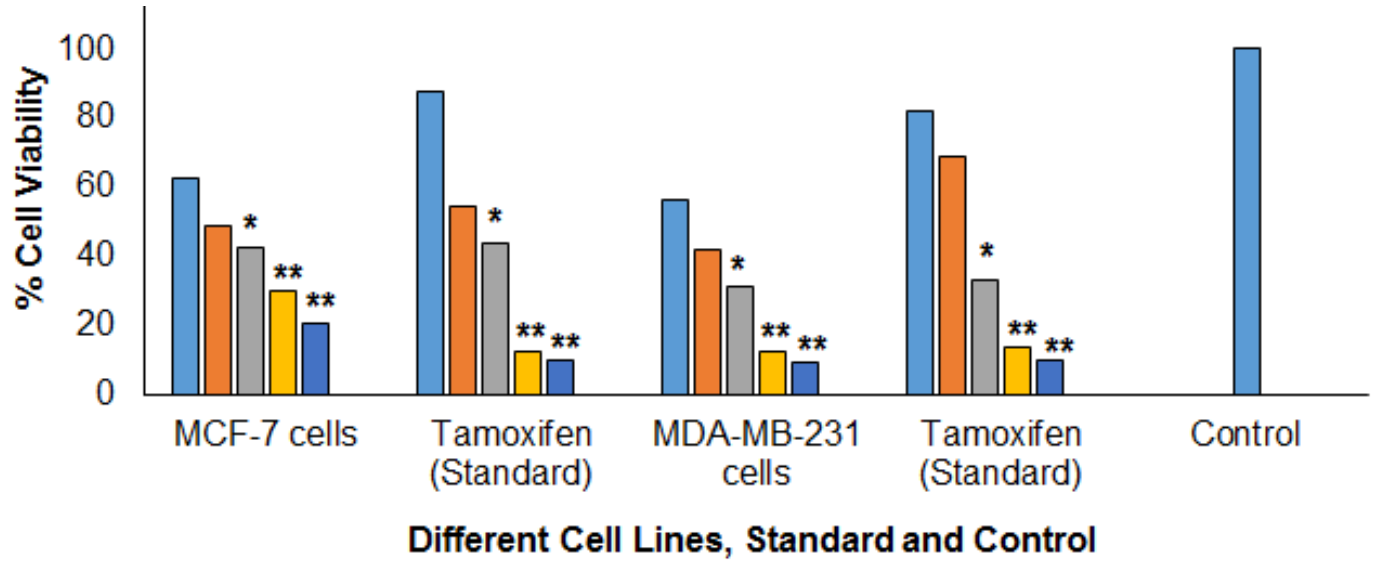
The contribution of new and novel products from potential bioactive plants or their extracts for disease treatment and prevention is still vast, despite the overshadowing by recent synthetic chemistry as a method of drug discoveries and drug productions (Kviecinski et al, 2008). Moreover, plant derived drugs like vinblastine, vincristine, taxol, and camptothecin had

Table-1. Percentages of the MCF-7 and MDA-MB-231 cell viability after the treatment with *Artocarpus heterophyllus* methanolic crude extracts at different concentrations.

Conc. (µg/mL)	% Cell Viability against different cell lines after the treatment with *Artocarpus heterophyllus* plant crude extracts			
	MCF-7 cells	Tamoxifen (Standard)	MDA-MB-231 cells	Tamoxifen (Standard)
62.25	62.91±0.44	87.7±0.11	56.65±0.27	82.40±0.11
125	48.68±0.45**	54.38±0.04	41.79±0.92*	68.90±0.01
250	42.82±1.43**	44.00±0.01*	31.36±0.26**	33.00±0.08**
500	29.69±0.67**	12.29±0.02**	12.30±1.33**	13.80±0.02**
1000	20.81±0.36**	10.1±0.01**	9.05±0.30**	10.20±0.01**

(*The cell viability was measured by the MTT assay. Control was the untreated cells. * and ** represented levels of significance at p < 0.05 and p < 0.01).*

Figure-1. Percentages of the MCF-7 and MDA-MB-231 cell viability after the treatment with selected medicinal plants methanolic crude extracts at different concentrations. (*The cell viability was measured by the MTT assay. Control was the untreated cells. * and ** represented levels of significance at p < 0.05 and p < 0.01).*

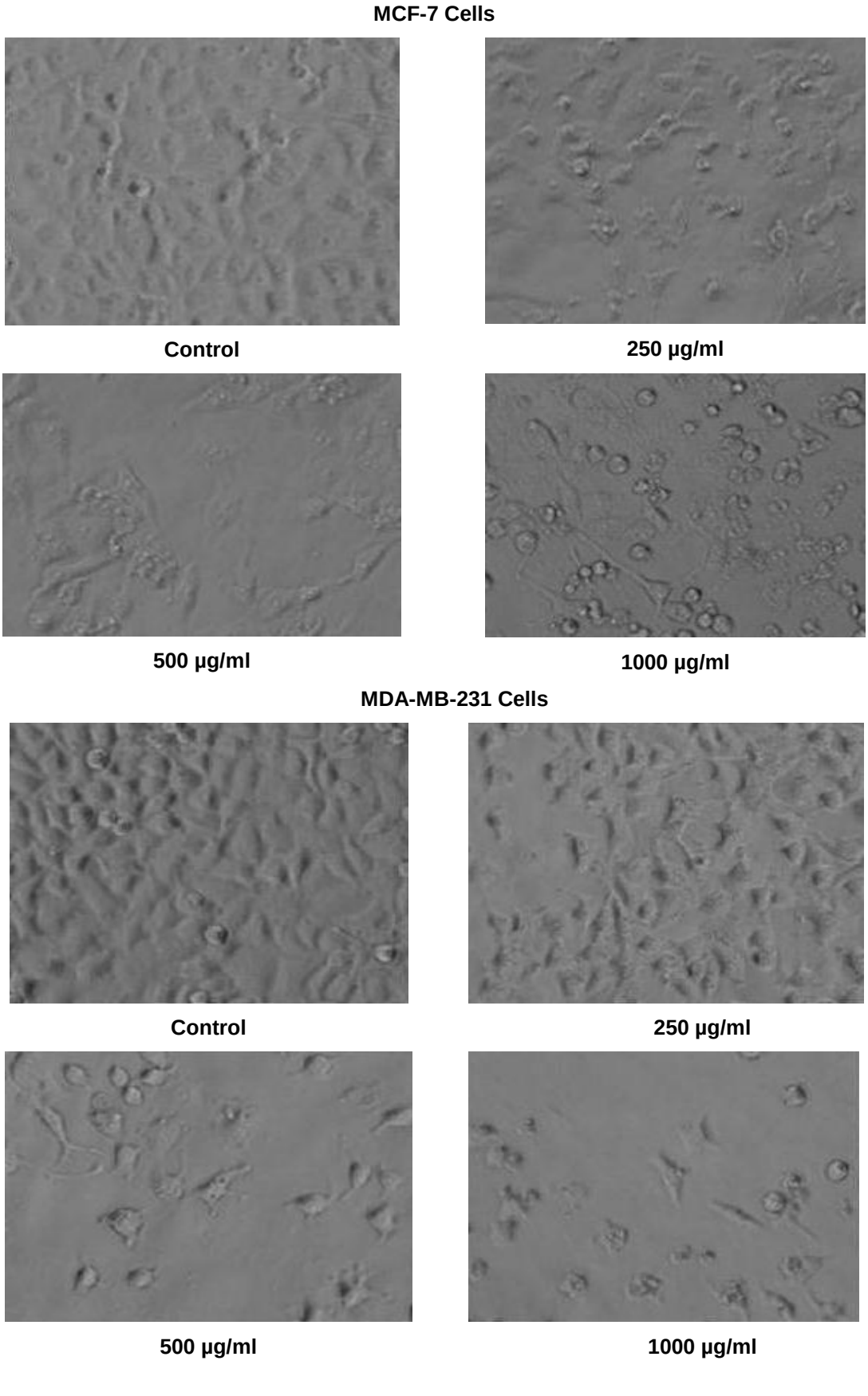


lead to greatest extend within the vicinity of antitumor upon where, the drugs were reported to improvise the chemotherapy of some cancers (Yousefzadi et al, 2010). Plants contain almost unlimited capacity to generate compounds that fascinates researchers in the quest for new and novel chemotherapeutics (Reed et al, 2005). The persistency search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention (Yan et al, 2009; Veenavani Marka et al, 2013). Numerous compounds found in plants with anticancer properties are such as alkaloids, phenylpropanoids, and terpenoids (Kintzios et al, 2006). Therefore, in this study the leaves extract of *Artocarpus heterophyllus* was evaluated as new anticancer agent by using MTT assays.

% Cell viability against MCF-7 cells

The anti-proliferative effect of *Artocarpus heterophyllus* methanolic crude extracts was tested on a cultured MCF-7 breast cancer cell line. Comparisons of the cell growth for 24h with various concentrations of selected medicinal plants leaf methanolic crude extracts (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml) are shown in Table-1 and Figure-1. *Artocarpus heterophyllus* showed significant high growth inhibitory effects on the MCF-7 cell line in a dose-dependent manner ($p<0.05$). *Artocarpus heterophyllus* inhibited the proliferation of MCF-7 cells with the viability percentages of 63% (62.5 µg/ml), 49% (125 µg/ml), 30% (500 µg/ml) and 21% (1000 µg/ml) compared to the vehicle treated blank. The crude extracts of this plant at 62.5 µg/ml did not significantly induce cell death ($p>0.05$, data not shown) comparing the control group. *Artocarpus heterophyllus* plant methanolic crude extract significantly had cytotoxic effects to the MCF-7 cells ($p<0.05$) comparing to the control group. This plant extract at 1000 µg/ml showed the highest cytotoxic effect (20.81±0.36% of viable cells) with IC_{50} 119 µg/mL. Therefore, it was clear that the methanolic crude extract of *Artocarpus heterophyllus* leaves had anti-proliferative effects on MCF-7 cells.

Figure-2. **Morphological changes in MCF-7 and MDA-MB-231 cells exposed to various concentrations of *Artocarpus heterophyllus* methanolic extract for 24 h.** (Images were taken using an inverted phase contrast microscope at 20X).



% Cell viability against MDA-MB-231 cells

The anti-proliferative effect of selected medicinal plant methanolic crude extracts was tested on a cultured MDA-MB-231 breast cancer cell line. Comparisons of the cell growth for 24h with various concentrations of selected medicinal plant leaf methanolic crude extracts (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml) are shown in Figure-2 and Table-2. *Artocarpus heterophyllus* inhibited the proliferation of MDA MB 231 cells with the viability percentages of 56% (62.5 µg/ml), 41% (125 µg/ml), 31% (250 µg/ml), 12% (500 µg/ml) and 9% (1000 µg/ml) compared to the vehicle treated blank. The crude extracts of all plants at 62.5 µg/ml did not significantly induce cell death ($p>0.05$, data not shown) comparing the control group and showed more than 50% viability. This plant extract at 1000 µg/ml showed the highest cytotoxic effect (9.05% of viable cells) with IC_{50} 94 µg/mL. The % of cytotoxicity (9%) showed by the *Artocarpus heterphyllus* plant was nearer to the value (10%) of Standard drug. Therefore, it was clear that the methanolic crude extract of *Artocarpus heterophyllus* had anti-proliferative effects on MDA MB 231 cells.

Morphological analysis of MCF-7 and MDA-MB-231 cell lines

The morphological changes in MCF-7 and MDA-MB-231 cells exposed to various concentrations of *Artocarpus heterophyllus* methanolic extracts are shown in Figures-3. Alterations in the morphology of MCF-7 and MDA-MB-231 cells were observed under phase contrast inverted microscope. Results showed that MCF-7 and MDA-MB-231 cells exposed to 500 and 1000 µg/ml concentrations of all extracts for 24 h reduced the normal morphology of the cells and cell adhesion capacity in compared to the control. Most of the cells at 1000 µg/ml of extract lost their typical morphology and appeared smaller in size, shrunken, and rounded. However, at 250 µg/ml of extracts did not cause any effects on the morphology of the cells.

Ohmagari and Berkes (1997) state that the methanolic extracts of *Artocarpus heterophyllus* bark leaf and bark extracts showed effectiveness against Bacillus subtilis and Pseudomonas fluorescens while aqueous extracts did not show considerable activity against test organisms. In addition to the antimicrobial activity of *A. heterophyllus*, anti-inflammatory, anti-oxidant, anti-cholinergic, anti-diabetic, immune modulatory effect, inhibition of protease, oestrogen regulation and inhibition of melanin biosynthesis have also been reported through several pharmacological research investigations of the plant parts (Padma et al. 1985).

Artocarpus heterophyllus Lam shows the *in vitro* anti-inflammatory effects of phenolic compounds isolated from the ethyl acetate extracts of the fruits of *Artocarpus heterophyllus.* Three phenolic compounds were characterized as artocarpesin (5, 7,2',4'-tetrahydroxy-6-βmethylbut-3-enyl) flavones), norartocarpetin (5, 7, 2',4' tetra-hydroxyflavone) and oxyresveratrol [trans-2, 4, 3',5'tetrahydroxystilbene] by spectroscopic methods and through comparison with data reported in the literatures (Rahman et al., 1999; The Wealth of India, 985). The anti-inflammatory effects of the isolated compounds were evaluated by determining their inhibitory effects on the production of proinflammatory mediators in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells.

The results of cytotoxicity study of methanolic extract of *Artocarpus heterophyllus* showed significant cytotoxicity against MCF-7 and MDA-MB-231 cell lines. This methanolic extract of *Artocarpus heterophyllus* is non-toxic to normal cells, but showed excellent toxicity on cancer cells. The cytotoxicity of methanolic extract of *Artocarpus heterophyllus* may be due to the presence of flavonoids having mono to poly phenolic groups in the structure. The flavonoids have reported for their cytotoxic activity due to presence of phenolic groups (Matsuo M., 2005).

CONCLUSION

From the results obtained from MTT assay, by the comparison of the IC_{50} values and linearity of the activity, the methanolic extract of *A. heterphyllus* showed excellent cytotoxicity against the MCF-7 and MDA-MB-231 cell lines. Cytotoxic changes observed was cell smaller in size, shrunken, and rounded aggregation. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of leaves of *Artocarpus heterophyllus* as an anti-cancer agent.

Competing interests

The authors have declared that no competing interests exist.

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