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EFFECT OF *SOLANUM CHRYSOTRICHUM* SCHLDL. ON CELL PROLIFERATION *IN VITRO*

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Keywords:

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ABSTRACT: The effect of four extracts of *Solanum chrysotrichum* on the proliferation of bacteria, yeasts, mouse spleen cells and human transformed cells was determined. Dry leaves grounded were consecutively macerated 24 h with hexane, chloroform, methanol and water. Dilutions of 1, 10 and 100 µg/mL / DMSO were used for the assays with normal mouse spleen cells, DU-145 and MCF-7 cells. The cellular proliferation was evaluated by the sulforhodamine method´s. Antibacterial and antifungal activities were determined by methods of diffusion and dilutions, using concentrations of 10, 15, 20 and 25 mg/mL. The bacteria used were *Escherichia coli* SOS, *Proteus mirabilis* NCTC2896, *Staphylococcus aureus* ATCC6538 and *Bacillus subtilis.* The yeasts were *Candida albicans*, *Candida tropicalis* and *Cryptococcus neoformans*. The bacteria were cultured in Agar-Mueller-Hinton and the yeasts in Sabouraud Agar and incubated at 37 and 28 ºC, respectively 24 h. Methanol and watery extracts at dosage assayed caused a cellular concentration reduction average of 56 and 59%, on cells DU-145, respectively. The chloroform extract at doses of 100 µg/mL increased to 40%, the concentration of mouse spleen cells, the other extracts had a cytostatic effect. This extract also reduced to 53% concentration of MCF-7 cells at 10 and 1 µg/mL. All concentrations of methanol extract inhibited to *E. coli* SOS and the concentration of 25 mg/mL also inhibited to *C. neoformans*. The aqueous extract inhibited the growth of *B. subtilis* with all concentrations assayed and to the other bacteria and *C. neoformans* with the 25 dose of mg/mL.

INTRODUCTION: *Solanum* is a large genus of herbs, shrubs, and rarely trees, found throughout the temperate and tropical parts of the world. It includes about 1500 species [1]. In Mexico, *S. torvum*, *S. lanceolatum* and *S. chrysotrichum* are popularly known as "sosa", and they are employed in the treatment of several skin mycosis conditions, particularly athlete´s foot, and cancer [2].

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Solanum chrysotrichum (Solanaceae*)* is a shrub or small tree of 4 m, pubescent with stellate hairs, stems rusty, with alternated leaves, broadly ovate, the lamina is up to 40 cm long and 30 cm wide, with thorns in the sheaf and in the back. This plant is distributed widely and abundantly from the south of Mexico to Panama. It grows to an altitude of 2000 to 2500 meters over the sea level. Is widely distributed from Veracruz to highlands of Chiapas, Mexico, were the leaves of this specie or from *S. torvum* are employed as folk remedy against ulcers, wounds, burns. The decoction of its leaves is taken to treat different types of cancer [3]. *Solanum* species have been a source of diverse of steroidal saponins [4, 5, 6].

It is well known that such compounds confer to the plant toxicity against other plants and protect itself from the attack of different pathogens and predators [7]. Saponins also have hemolytic, mollusquicidal, anti-inflammatory, antimicrobial, cytotoxic and antitumor activities *in vitro* and *in vivo* [8].

In our previous work, we reported the antibacterial, hematopoietic and cytotoxic properties of *Solanum torvum in vitro*. Considering that *S. torvum* and *S. chrysotrichum* have the same geographical distribution, ethnomedicinal applications and named indistinguishable as sosa, being different species [9]. The goal of this study is to evaluate the effect of organic and aqueous extracts from the leaves of *Solanum chrysotrichum* on the proliferation of normal hematopoietic mouse cells, MCF-7, DU-145 cells from human breast and prostate carcinoma, respectively, as well as the growth of bacteria and yeasts.

MATERIALS AND METHODS:

Plant Material: Aerial parts of the plant were collected by one of the authors (Rodolfo Velasco) in Ahila, Puebla, on September 2015, and authenticated by Dr. Adolfo Espejo Serna from the Herbario Metropolitano "Ramón Riba" of the Universidad Autónoma Metropolitana (UAM) Iztapalapa, where a voucher specimen of the plant (55771) is stored.

Preparation of Extracts: The aerial parts were dried at room temperature, protected from dust and sunlight. The leaves were grounded; 500 g of such material was macerated with 3 L of hexane 3 L, 24 hat room temperature. The extract was filtered, with the retained plant material the process was repeated using chloroform, methanol or water. The organic solvents were evaporated to dryness under reduced pressure, at 35 ºC in a rotavapor Buchi RII (Swizerland). Two grams of aqueous extract were dissolved with water, and fractioned with 200 ml of the following solutions; hexane, hexane-ethyl acetate, (v/v), ethyl acetate, ethyl acetate-chloroform (v/v), chloroform, chloroform-water (v/v).

Phytochemical Screening: A preliminary phytochemical study of methanol and watery extracts was performed by coloring and precipitation assays as reported [10]. Proteins were determined by the Lowry´s method [11].

Experimental Animals: mice DC1, of 8-12 weeks -old from the UAM-Iztapalapa animal facilities were used. Four mice per box were housed at a constant temperature of 24 ºC with a 12 h light / 12 h darkness photoperiod and allowed free access to food and sterilized water by filtration through 0.22 µm Millipore membranes (USA). The handling of laboratory animals and experimental procedures were performed according to the national and international rules (NIH guidelines for handling and care for animals), including the Official Mexican Rule [12]. Also, the study was approved by the ethical institutional committee.

Assays for Cytotoxic Activity:

Human Transformed Cell Lines: Human prostate carcinoma (DU-145) and human breast carcinoma (MCF-7) cells were cultured separately in RPMI-1660 medium supplemented with 10% newborn calf serum heat inactivated (*in-vitro*, Mex). The cells were grown at 37 ºC to confluence in a CO_2 incubator (National, USA). One hundred and ninety µl containing 4.5×10^5 cells/mL in RPMI-1640 media - 10% newborn calf serum inactivated (*In vitro*, Mex.) were added to 96-well plates culture (Nunc, USA). The plates were incubated 24 h at 37 ºC in a CO_2 incubator, after this time 10 µl of hexane, chloroform, methanol or watery extracts were added to the wells. The extracts were previously dissolved in 10% dimethylsulfoxide [(DMSO) (Sigma Chem, Saint Louis, MO)] distilled water, added to the cultures at final concentration of 1, 10, and 100 µg/mL of each extract, then the plates were incubated 48 h at 37 ºC in a CO_2 incubator. Each extract was tested five times in triplicate independent experiments [13]. To evaluate the cell proliferation the sulforhodamine B (SRB) method was used. ED_{50} <50 µg/mL [14].

Hematopoietic Activity in Spleen Cultures: In order to know the effect of extracts on the proliferation of normal cells, cultures of hematopoietic cells from spleen were performed. Mice were sacrificed in a CO_2 chamber, the spleen was isolated, placed on a metal mesh and mechanically dispersed using tweezers and scissors. α-MOPS medium [(Minimum Essential Medium-/3-[-Morpholino]-propane sulfonic acid)],

(Gibco-BRL) 30 ml were added to10% heat inactivated horse serum (*In vitro* - Mex), cells were washed with α-MOPS medium, and centrifuged at 3500 rpm for 15 minutes at room temperature. The cell pellet was suspended in α-MEM medium (Sigma, Saint Louis, MO) (pH 7.O), supplemented with 10% horse serum. Cells were counted in a hemocytometer, and cell viability determined using 0.2% trypan blue. Cell concentration was adjusted to 2.0×10^6 cells/mL.

One volume of this suspension was added to 6 volumes of α-MEM medium, 2 volumes of heat inactivated horse serum (Sigma, Saint Louis, MO), and 1 volume of extract to adjust to 1, 10, 100 µg/mL. Then, 0.50 ml of this mixture was placed (duplicate) in 24 - well plates (Nunc, USA) and incubated at 37 °C for 48 hours in a wet atmosphere with 5% CO_2 and 95% air. Cells were then harvested, washed with PBS (pH 7.2), centrifuged twice at 3000 rpm, and finally suspended in 0.5 ml PBS (pH 7.2). Cells were counted with a hemocytometer [15]. Each experiment includes extract free cultures. Results were expressed as mean ± standard error. The extract-treated cultures were compared to control cultures using ANOVA variance analysis.

Antimicrobial Activity: The antibacterial and antifungal effects were conducted by the diffusion (Kirby Bauer) and dilution methods, for the first one, concentrations of 1, 10 and 100 µg/mL were tested, and 10, 15, 20 and 25 mg for method of dilutions, respectively. The used bacteria were ATTC8937 *Escherichia coli,* SOS *Escherichia coli,* ATTC6538 *Salmonella typhi,* NCTC2896 *Proteus mirabilis,* ATTC6538 *Staphylococcus aureus, Bacillus subtilis,* and the yeasts *Candida albicans, Candida tropicalis* and *Cryptococcus neoformans.*

The bacteria were cultured in Mueller-Hinton Broth (Bioxon-Mex) and incubated for 18 hrs at 37 °C, while the yeasts were cultured in Sabouraud Dextrose Broth 20 h at 28 °C. After then, the cell concentration was adjusted 3×10^8 CFU/mL (1 McFarland nephelometer tube/Barry and Thorns-berry, An antibiotic solution (10^4 IUPenicillin-10^4 µg/mL Streptomycin, Sigma, Saint Louis, MO) was used as positive control for bacteria, and a 1X Antifun solution (Amphotericin B 250 µg/mL, Microlab, Mex.) for yeasts.

Also, a negative control with physiological saline solution was included as non-inhibition control. Bacterial cultures were incubated at 37 °C and yeasts cultures at 28 °C for 24 h [16]. *B. subtilis*, *Escherichia coli* SOS and the yeast were donated by National School of Biological Sciences of the National Polytechnic Institute.

RESULTS:

Phytochemical Analysis: In analysis of methanol and watery extracts tannins, sugars and saponins were obtained. No flavonoid, triterpene, or alkaloids were detected. **Table 1** shows the yield and total proteins of each extract 500 grams of initial material.

TABLE 1: RECOVERY AND TOTAL PROTEINS CONTENT OF EXTRACTS

Extract	Recovered (g)	Yield (%)	Proteins µg/mL
Hexane	5.7	1.15	0.00
Chloroform	4.5	0.92	6.84
Methanol	13.5	2.70	29.00
Water	44.5	8.90	38.00

Cytotoxic Activity on Human Transformed Cells: Human Prostate Carcinoma Cells (DU-145): Fig. 1 Hexanic extract at doses of 1 µg/mL reduced 43% of the cell viability, meanwhile, 10 and 100 µg/mL acted asstimulant and cytostatic of the cell proliferation, respectively. Chloroformic extract reduced proportionally the survival in respect of the dose tested $p<0.001$. Methanolic extract was cytotoxic in all doses. The difference of the activity between the three doses is statistically significant with $p<0.01$. Doses of 1 and 10 µg/mL of the watery extract were able to kill more than 50% and 70% of the cultured cells, respectively with $p<0.001$. This extract had the highest cytotoxic activity.

Fig. 2 Doses of 1 and 10 µg/mL of the hexanic extract diminished an average of 56% of the cell concentration, whereas a dose of 100 µg/mL stimulated cell proliferation, reaching 82% of the increase. Chloroformic extract at doses tested diminished 45% of the concentration of cells without difference among them. Doses of 1 and 100 µg/mL of methanolic extract of reduced 30% concentration of survival cells, whereas 10 doses of µg/mL behaved as cytostatic, the difference between the activities of this doses with respect to the other two is statistically significant at $p< 0.001$.

With doses of 1 and 10 µg/mL of watery extract, the cellular population was reduced to 47% and 56%, respectively. No statistically significant difference between of both doses. On the contrary, the 100 dose produced an increase of 20% in the concentration of cells.

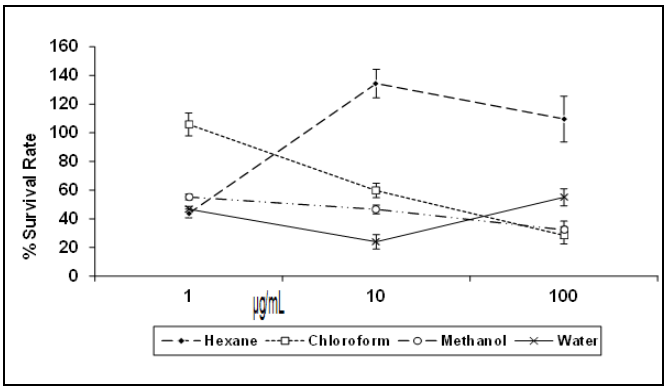

FIG. 1: CYTOTOXIC ACTIVITY OF *SOLANUM CHRYSOTRICHUM* EXTRACTS ON DU-145 CELLS
Data are expressed as mean ± standard error p<0.001.
Cell lines: DU-145 human prostatic carcinoma

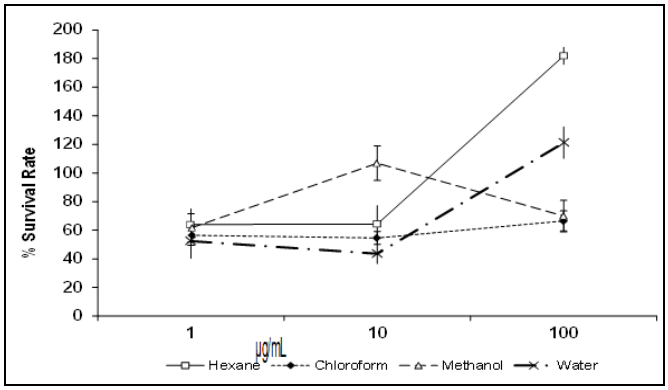

FIG. 2: CYTOTOXIC ACTIVITY OF *SOLANUM CHRYSOTRICHUM* EXTRACTS ON MCF-7 CELLS
Data are expressed as mean ± standard error. p< 0.001.
Cell line: MCF-7 human mammary carcinoma

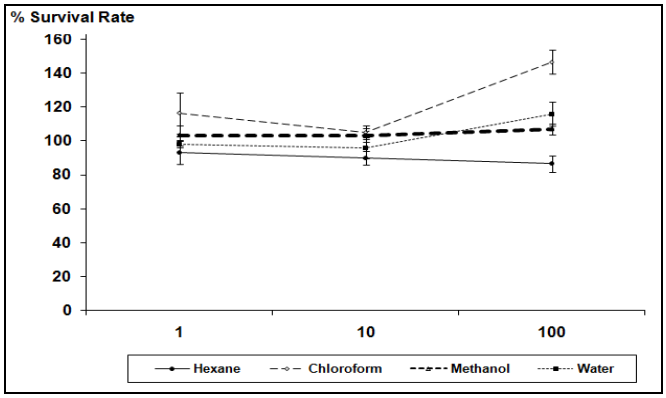

FIG. 3: HEMATOPOIETIC ACTIVITY OF *SOLANUM CHRYSOTRICHUM* EXTRACTS ON NORMAL SPLEEN MOUSE CELLS Data are expressed as mean ± standard error. p<0.001

Fig. 3 shows that hexane extract reduced 7% and 14% of the cell population with the doses assayed, which means that it is acting as cytostatic material (no toxic). With the chloroform extract increase of 16%, 4% and 46% of cell population were observed with 1, 10 and 100 µg/mL, respectively, p<0.001. With extract of methanol, the cell population increased from 3 to 7%, with the tested doses. The aqueous extract at doses of 1 and 10µg/mL kept the cell population constant, a dose of 100 µg/mL increased 16% of the cell population. Its stimulating activity is statistically significant with respect to the other two doses at p<0.001.

Antimicrobial Activity:

Kirby-Bauer Method (Diffusion): Concentrations of 1, 10 and 100 of hexanic, chlorformic or methanolic extracts did not inhibit the growth of bacteria by the Kirby-Bauer (diffusion) method, only the watery extract at a dose of 100 µg/mL inhibited the growth of the bacteria used, except *E. coli* SOS. This activity was confirmed when evaluating the crude extract along with its fractions, one of them, chloroform- water and the residual displayed antibacterial activity **Table 2**.

Method of Dilutions: Extracts of hexane and chloroform did not inhibit the growth of bacteria or yeasts. Nevertheless, extract of methanol in all tested doses inhibited the growth of *Escherichia coli* SOS and with the highest dose to *Cryptococcus neoformans*. The watery extract in all the dose was inhibited to *Bacillus subtilis*, a dose of 25 mg/mL inhibited to all bacteria and the yeasts *Candida tropicalis* and *Cryptococcus neoformans*.

TABLE 2: ANTIBACTERIAL ACTIVITY OF FRACTIONS FROM THE WATERY EXTRACT OF *SOLANUM CHRYSOTRICHUM* ASSAYED BY THE METHOD OF DIFFUSION

Microorganism	Raw Extract µg/mL			Chloroform/ Water v/v µg/mL			Residual Fraction µg/mL		
	1	10	100	1	10	100	1	10	100
Bacillus subtilis	-	-	+	-	+	+	+	+	+
Escherichia coli SOS	-	-	-	-	-	+	-	-	+
Escherichia coli ATTC8739	-	-	+	-	+	+	+	+	+
Salmonella typhi ATTC 6539	-	-	+	-	-	+	+	+	+
Staphylococcus aureus ATTC 6538	-	-	-+	-	+	+	+	+	+
Proteus mirabilis NCTC 2896	-	-	-+	-	-	+	+	+	+

Grading of results: -, no inhibition; +, inhibition zone < 10 mm in diameter

TABLE 3: ANTIBACTERIAL AND ANTIFUNGIC ACTIVITIES OF METHANOL AND WATERY EXTRACTS OF *SOLANUM CHRYSOTRICHUM* TESTED BY THE METHOD OF DILUTIONS

Extract	Methanol (mg/mL)				Water (mg/mL)			
Microorganism	10	15	20	25	10	15	20	25
Bacillus subtilis	-	-	-	-	+	+	+	+
Staphylococcus aureus ATTC6538	-	-	-	-	-	-	-	+
Escherichia coli SOS	+	+	+	+	-	-	+	+
Escherichia coli ATTC8739	-	-	-	-	-	-	-	-
Salmonella typhi ATTC 6539	-	-	-	-	-	-	-	+
Proteus mirabilis ATTC 2896	-	-	-	-	-	-	-	+
Candida albicans	-	-	-	-	-	-	-	+
Candida tropicalis	-	-	-	-	-	-	-	+
Cryptococcus neoformans	-	-	-	+	-	+	++	+++

Grading of results: -, no inhibition; +, inhibition zone < 10 mm in diameter; ++, inhibition zone of 10 to 15 mm in diameter +++, inhibition zone of 16 to 20 mm in diameter

DISCUSSION: Some species of the genus *Solanum* have been studied for their anticancer properties, investigations have been conducted to evaluate *in vitro* the cytotoxic effect of raw extracts or compounds from *Solanum* on the proliferation of human carcinoma cells, as HeLa, that was inhibited by a methanolic extract of *Solanum nigrum* with an IC_{50} of 10 to 0.156 mg/mL[17], although such extract did not inhibit to VERO cells. In other studies, it has been showing that an ethanolic extract of *S. rostratum* affected the viability of VERO cells 80, 39, and 40% at 12, 24 and 48 hours of exposure, respectively [18].

In regards to our results on DU-145 cells, only the aqueous extract at 1 and 10µg/mL inhibited more than 50% of the proliferation of these cells. According to the international criteria for raw extract it would be a good candidate to continue the isolation of the compounds responsible for the cytotoxic activity. Other cell line used to test cytotoxicity is MCF-7 (breast carcinoma) that was exposed to an aqueous macerated pulp and pericarp from *S. melanogena*, showed that two alkaloids isolated from the pulp inhibited to the topo-isomerase I at concentrations of 50 and 100 uM [19].

In our study, watery and chloroformic extracts produced cytotoxicity higher than 50% on MCF-7 cells in concentrations of 1 and 10µg/mL. Although we worked with MCF-7 cells, it is not possible to compare the activity of both aqueous extracts, since we used a raw extract expressed in µg/mL, mean while, they used purified compounds expressing the activity in µM concentration [22]. Also, It has been reported [23] that an ethanol 60% extract of aerial parts of *Solanum trilobatiuim* was cytotoxic for cells MCF-7with an IC_{50} of 29.4 and 15.3 µg/mL for saponins M Torvoside and N Torvoside, respectively isolated by them [20].

It is important to emphasize that *S. chrysotrichum* and other solanaceous contain saponins and It is known that saponins pentacyclic or steroidal have hemolytic effect [8]. In our study, *S. chrysotrichum* did not have cytotoxic effect on erythroid cell precursors in spleen cultures, even more, 100 µg/mL concentration of the chloroformic extract stimulated the proliferation of spleen cells, while other extracts did not show cytotoxic effect. Some of our extracts acted as much as cytotoxic on the transformed cell but as cytostatic on hematopoietic cells in spleen cultures.

Previous studies about the effect of an aqueous extract of leaves of *S. hipidum* on hematopoiesis of mice *in-vitro* and *in-vivo* (results not included) showed that there was no toxic for bone marrow cells or spleen cells *in vitro*, and it not modified the level of blood cells four days after the oral administration of the extract [21].

Antibacterial and Antifungal Activities: Some studies have reported antibacterial and antifungal properties of species of *Solanum* genus. An ethanolic extract of the leaves of *S. trilobatum* inhibited the growth of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and the fungus *Aspergillus niger* and *Fusarium* sp. [23]. A methanol extract from leaves or seeds from *S. xanthocarpum* inhibited to *Aeromona hydrophila*, *E. coli*, *S. aureus*, *Salmonella typhi* and the fungus *Candida albicans*, *Aspergillus flavus* and *Trichophyton mentagrophytes* [22].

In our study concentrations of µg/mL 1, 10 and 100 extracts were used but only the aqueous extract (100µg/mL) presented antibacterial activity by Kirby´s Bauer method, such activity was improved in the chloroformic fraction of the aqueous extract since doses of 10 µg/mL was inhibited to *B. subtilis*, *E. coli* and *S. aureus.* These results are according to the one previously reported for a chloroformic-methanol extract from the roots of *S. torvum* containing oleic and linoleic acids among other compounds that inhibited bacterial growth [23]. Our results using the Kirby-Bauer`smethod showed poor or none activity compared to other reports possibly because we used concentrations of form1, 10 and 100 µg/mL while others employed concentration from 5 to 100 mg/mL.

Solanum chrysotrichum has been used in Mexican traditional medicine for the treatment of superficial mycotic infections as Tinea pedis, Pytiriais capitis and cervicovaginitis due to *Candida* species. Steroidal saponins from *S. chrysotrichum* denominated SC-1, SC-2 and SC-6 have shown antimycotic activity against *T. rubrum*, *Aspergillus niger* and *C. albicans* [24]. In the present study, the aqueous extract of *S. chrysotrichum* inhibited the growth of *Candida tropicalis* (mg/mL 25) and *Cryptococcus neoformans* at 15, 20 and 25 mg/mL, but not to *C. albicans* possibly because, we used a crude extract and by successive maceration that supposes with less rich in purified compounds than they used. Toxicology, genotoxicity and cytotoxicity of three extracts from *S. chrysotrichum* have been evaluated by hepatic biochemical tests (alanine amino-transferase (ALT) and aspartate aminotransferase (AST) and no significant differences were found in respect of the control group. Researchers reported moderate necrosis in liver, and focal tumefaction in kidney, significant but clinically irrelevant, also elevation of creatinine was observed with aqueous and hydro alcoholic extracts [25].

According tothe international criteria, hexane and watery extracts of *S. chrysotrichum* are cytotoxic for DU-145 and cytotstatic for MCF-7 cells, but not for the cells of the hematopoietic system, with makes difference other than plants or compounds that are generally cytotoxic. This plant is considered a noxious weed for crops and combated, but could be necessary to further detect possible immunological properties.

CONCLUSION: Hexane and watery extracts of *S. chrysotrichum* were cytotoxic for DU-145 and cytotstatic for MCF-7 cells, but not for normal mouse spleen cells. The concetration of 25 mg/mL of the aqueous extract presented antibacterial and antifungical activities.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

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