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Beneficial antibacterial, antifungal and anti-insecticidal effects of ethanolic extract of *Solenostemma argel* leaves

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Abstract: *Solenostemma argel* belongs to the *Asclepiadaceae* family, a desert plant wide spread in the centre and north of Sudan. *Solenostemma argel* leaves were soaked in 1000 ml of 80% ethanol with a 27.1% of yield. The phytochemical screening method was described and modified. The qualitative chemical screening of the crude and fraction extract to test the secondary metabolites showed the presence of alkaloids, flavonoids, terpenoids, triterpenes, saponnins and tannins.

The diffusion method has been used for four types of bacteria, two Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and two types of fungal *Aspergillus niger* and *Candida albicans*. Higher growth inhibition zone diameters were obtained from *Escherichia coli* , *staphylococcus aureus* , *pseudomonas aeruginosa* , *Aspergillus niger* and *candida albicans*.

The Second larval instars of African melon ladybird beetle were used in this study in order to assist the insecticidal effect of *Solenostemma argel* leaves. Different concentrations (1.5%, 2.5%, 5%) were applied and the percentage of mortality was observed at 24, 48 and 72 hours.

Keywords: Extraction; *Solenostemma argel;* Antibacterial; Antifungal; insecticidal Activities.

Introduction

Plant has been used in treating human diseases for thousands of years. In certain African countries, up to 90% of the population still relies exclusively on plant as source of medicines [1]. Environmental degradation provides threats to biological diversity but the sub Saharan region still boasts wide variety of indigenous species. There are considerable economic benefits in development of indigenous medicines and use medicinal plants for the treatment of various diseases [2]. Sudan is a rich country with indigenous herbal resources. This is due to the variation in climate, rainfall and soil. This variation allowed the growth of a large number of medicinal plants [3]. *Solenostemma argel* belongs to *Asclepiadaceae* family, it has many velvety pubescent branches at the base in the northern region [4]. Locally known for its benefits, it is widely used in Sudanese traditional folkloric medicine as antispasmodic [5], anti-inflammatory and anti-rheumatic agent [6]. This plant is used in the treatment of hypercholesterolemia, diabetes mellitus, cold cough, jaundice and measles [7]. The plant also possesses insecticidal effect and hence was used to combat insect pests [8]. Moreover, it was reported to have antimicrobial properties as well as antibacterial and antioxidant activity [9]. Most of Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases [10]. Argel (*Solenostemma argel* (Del) Hayne), or locally called "Hargal" is an erect perennial under-shrub that reaches up to 1.5–2 feet in height with numerous branches carrying opposite decussate leaves. The leaves are lanceolate to oblong-ovate, with acute or sub–acute apex and cuneate base. The leaf petiole is thick [7]. Fruits are solitary follicles, thick, ovoid, lanceolate, acuminate at the apex and very hard with dark purple color. Seeds are turgid, ovoid and channel down at one face. They are minutely tuberculate bearing an apical tuft hair [11]. *Solenostemma argel* is a desert plant, widely spread in Central and North parts of Sudan, Egypt, Libya, Chad, Algeria, Saudi Arabia and Palestine. However, Sudan is regarded as the richest source of this plant.

Elkamali [11] conducted a phytochemical screening of the constituents of argel (*Solenostemma argel*) leaves, stems and roots at the pre-flowering and flowering stages. Results showed presence of a number of chemical groups *(Flavonoides, tannins, sterols, triterpens and saponins*) with the major constituents being saponins. The bioactive effects of Hargal plant are mainly attributed to the presence of a

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variety of organic substances mainly (teroenes, pergenine, glycosides, and sterols) [12].

The insecticidal activity of *Solenostemma argel* was investigated by many researchers in many countries [13]. Hag-Eltayeb *et al.* reported that argel aqueous extract was effective in the control of the larvae of mosquitoes *Culex spp* and *Anopheles spp* under laboratory conditions. In laboratory, aqueous and organic extracts showed mortality, repellency and anifeedant effects against cow pea beetle *Callosobruchus maculates* [14]. Sidahmed *et al.* [15] found that aqueous filtrates of Argel plant concentrated at 10% gave 100% mortality of workers and soldiers of the cotton soil termite (*Microtermes thoracalis* Sjost) under laboratory conditions. Furthermore, spraying Argel shoot water filtrate at 1ounce/6liter of water/tree was recommended to control white scale insect (*Parlatoria Blanchardii* Targ.) and (*Asterolicanium phoenicis)* on date palm [16]. Mardi and Suliman [17] found that the aqueous extract of Argel shoots at 40g/L of water gave comparable performance to the synthetic insecticide Alpha-cypermethrin.

Solenostemma Argel contains Flavonoids, triterbins, tannins, steroids, alkaloids, saponins, monoterpene, pregnanes, steroids lipids, flavones, antocinan-oxides, mucilages amino acids, polyholosides, polyphenolics, phytosterols and carotenoids. On the other hand, chemical investigations of leaves showed the presence of carbohydrates, protein, fiber and lower percentage of minerals K, Ca, Mg, Na, Cu, Fe, Mn, and non-nitrogenous protein [18-24]

Materials and Methods

Preparation of the plant material

Sample of leaves were obtained from El-Obeid market (March, 2015) and authenticated by plant herbarium at national research center Khartoum. The sample was allowed to dry at room temperature and then ground in to powder. Two hundred grams of the plant sample was extracted by soaking in 1000 ml of 80 % ethanol for about seventy-two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness. The yield percentage was calculated as followed:

$$\text{crude extract\%} = \frac{\text{weight of extract}}{\text{weight of sample}} * \mathbf{100}$$

Fractionation of ethanolic extract

20g of Ethanolic extract was dissolved in 500 ml of distilled water and shacked, three times with 100 ml of petroleum ether each time using separatory funnel. Ether layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layer was then re-shacked three times with 100 ml of chloroform in each time using reparatory funnel. Chloroform layers were combined together and evaporated under reduced pressure using rotary evaporator. Aqueous layer was then re-shacked, three times with 100 ml of ethyl acetate in each time using reparatory funnel. Ethyl acetate layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layer was finally shacked, three times with 100 ml of n-butanol in each time using reparatory funnel. Butanol layers were combined together and evaporated under reduced pressure using rotary evaporator apparatus. Aqueous layer was lyophilized using freeze-drier machine till dryness and the yield percentage of each fraction was calculated.

Figure 1. Solenostemma argel

Assessment of antimicrobial activity:

The cup-plate agar diffusion method was adopted with some modifications to assess the antibacterial activity of the prepared extract [25].

The averages diameter of growth inhibition zones are compared to standard chemotherapeutic agent. We have used five dilution concentration of the plant extract: 100, 50, 25 12.5 and 6.25 mg/ml.

Assessment of antifungal activity:

The antifungal activity of the above cited extracts was determined using a standard agar well diffusion method. We chose two standards fungi; *Aspergillus Niger* and *Candida albicans* . In this well-known procedure, agar plates are inoculated with a

standardized inoculum of the tested microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. The diameters of inhibition growth zones are measured and compared to standard antifungal agent.

Assessment of anti-insecticidal activity

The melon ladybird beetle (Henosepilachna elaterii) is a phytophagous ladybird species found in southern Europe, Africa and western Asia (Figure 2). The larvae and the adult feed on leaves and fruit of cucurbites, melon in particular. The highest damage is caused by larvae.Adults and larvae feed on the leaf surface, scraping away cells to form open windows, causing the leaf to wither. Extensive feeding can completely skeletonize the leaf. They can sometimes also feed on the fruit causing surface damage through which secondary infection may occur.

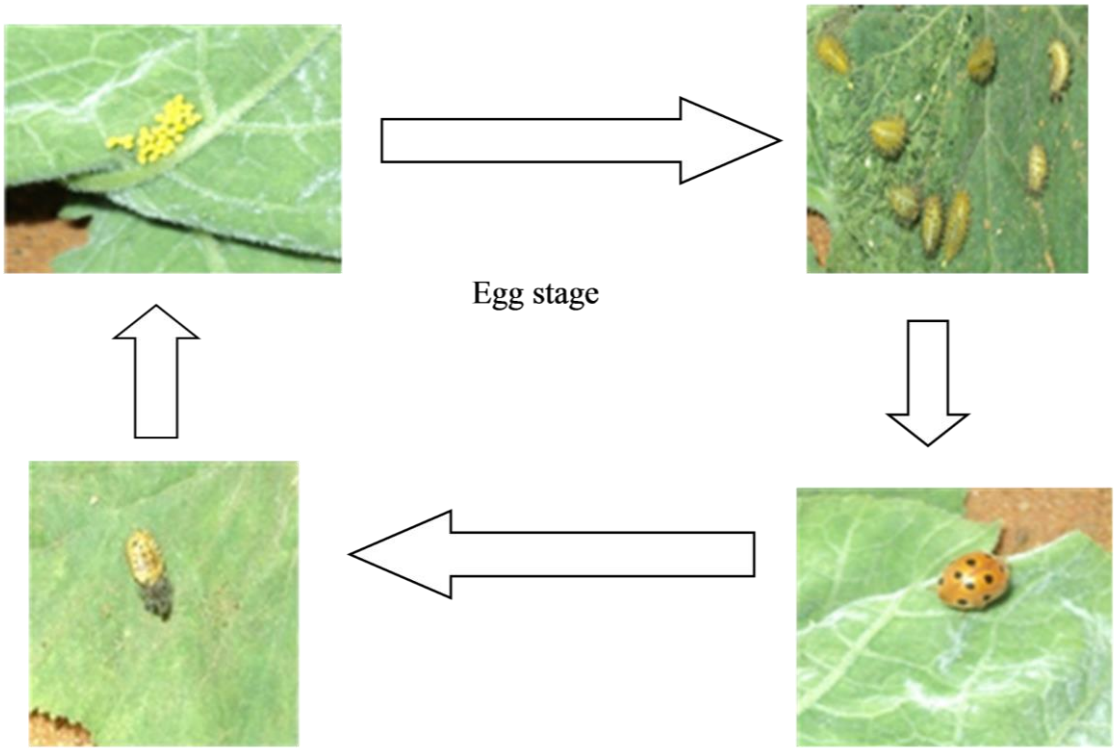


Figure 2. African melon ladybird beetle

The dilution of the extracts is prepared in the following concentrations: 1,25%, 2.5% and 5%. One piece of filter paper was kept in the petri dish and 2 ml of the drug was poured over it; then dried over 24 hrs. Larval instars were placed in each of the petri dish and their and mortality is monitored. Cypermetherin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects, here it was used as standard and methanol as a control. All these were kept without food for 24 hours. The insects were observed at intervals for 24 hrs.

Results and Discussion

The presence of various types of secondary metabolites of *Solenostemma argel* leaves were reported in Table 1. The results showed that Saponins and Cumarines are found in low concentration, Alkaloids, Tannins, Flavonoids and Steroids in medium concentrations, but Anthraquinones were no detected. The phytochemical screening of the fraction extract (Ethyl acetate) is shown in Tables 1. The results showed that Saponins and Alkaloids are found in low concentration, Coumarins, Tannins and Steroids in medium concentration, the Flavonoids and triterpenes showed higher concentrations, but the Anthraquinones was not detected.

Table 1. Phytochemical screening of the crude extract and Fraction extract (Ethyl acetate).

Components	crude extract	Fraction extract (Ethyl acetate)
Saponins	+	+
Cumarines	+	+
Alkaloids	+ +	+ +
Tannins	+ +	+ +
Flavonoids	+ +	+ +
Steroids	+ +	+ + +
Triterpenes	+ + +	+ + +
Anthraquinones	-	-

+++ means high concentration ++ means medium concentration + means low concentration _ means no detectable

Assessment of Antimicrobial Activities:

Plant extracts at the concentration of 100, 50, 25, 12.5 and 6.25 mg/ml were used for four bacteria (E.c, P.s, S.a, B.s) and the inhibition zone are shown in Table 2. The results indicate a negative activity of Petroleum ether and water extract against the four bacteria for the five concentrations. Ethyl acetate also is ineffective against B.s at all the analyzed concentrations.

Table 2. Preliminary screening for antimicrobial activity of *Solenostemma argel* Leaves against standard organisms (E.c) *Escherichia coli*, (p.s) *Pseudomonas aeruginsa*, (S.a) *Staphylococcus aureus*, (B.s) *Bacillus subtilis*.

	Conc mg/ml	Inhibition zone (in mm)			
		E.c	**P.s**	**S.a**	**B.s**
Ethanol extract	100	14	13	12	11
	50	16	13	12	10
	25	14	13	14	11
	12.5	13	10	15	10
	6.25	10	8	11	8
Chloroform extract	100	14	18	13	15
	50	15	15	13	13
	25	15	13	13	12
	12.5	13	13	17	10
	6.25	11	9	11	9
n-Butanol extract	100	17	17	15	15
	50	13	15	12	11
	25	13	15	13	14
	12.5	13	15	15	14
	6.25	12	13	10	13
Ethyl acetate	100	19	18	19	17
	50	17	18	18	-
	25	19	15	19	-
	12.5	19	13	13	-
	6.25	10	10	11	-
Petroleum ether	100	-	-	-	-
	50	-	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
Water extract	100	-	-	-	-
	50	-	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-

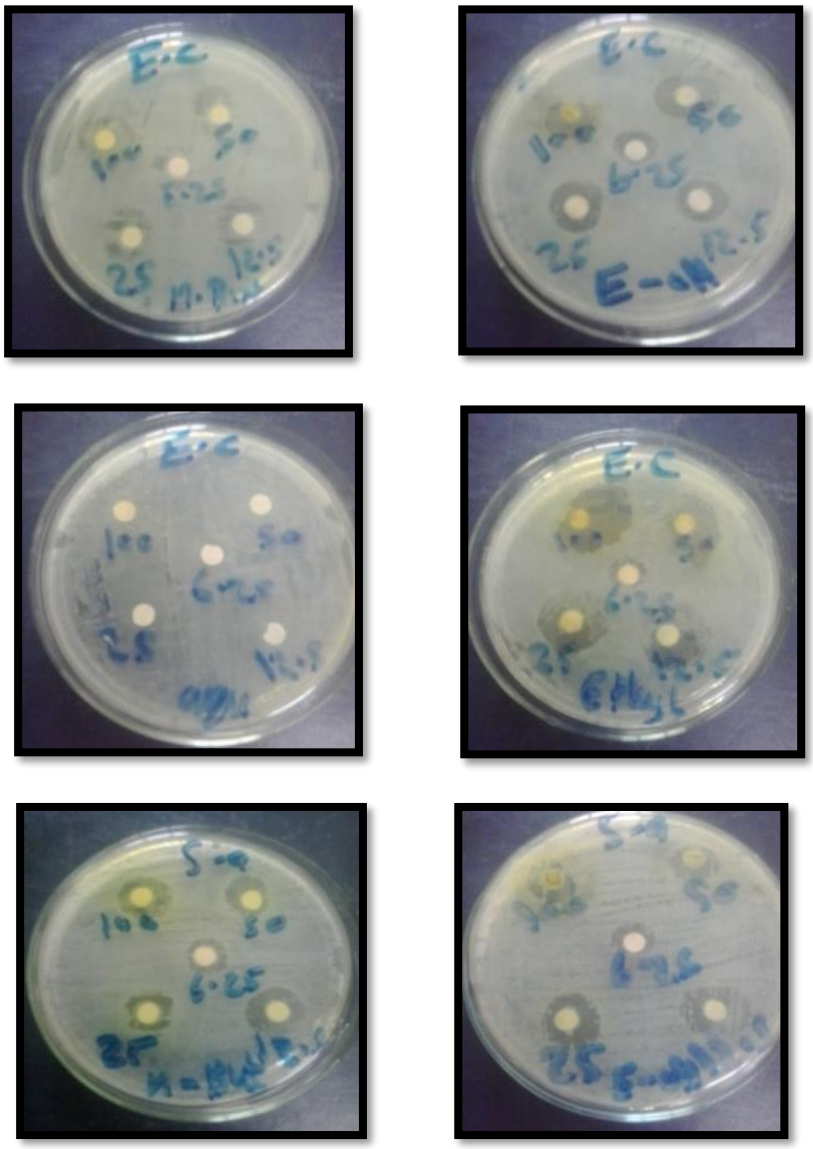
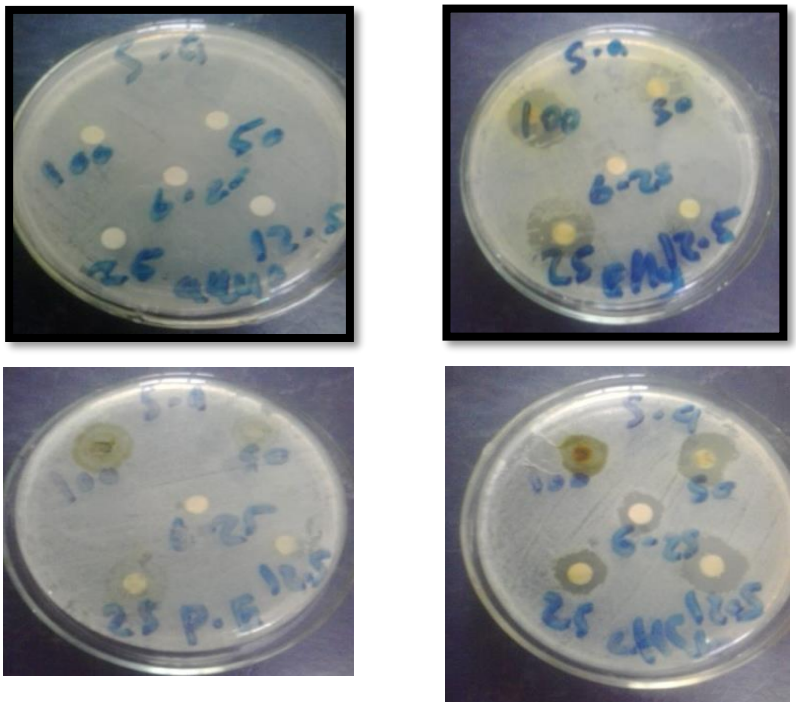


Figure 3. Inhibition zone of bacteria (Escherichia *coli)*



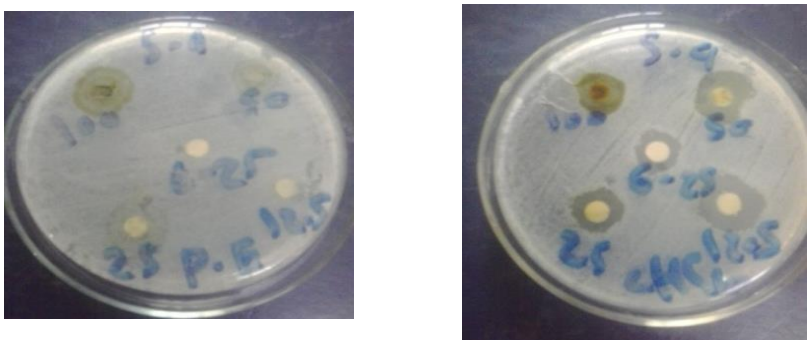


Figure 4. Inhibition zone of bacteria *(Staphlococcus aureus*)

We assisted the antifungal activity of our extracts againt *candida albicans* and *aspergillus niger* and the results are shown in Table 3 below.

Table 3. Preliminary screening for antifungal activity of *solenostemma argel* (leaves) extracts against standard organisms (C.a) *Candida albicans*, (A.n) *Aspergillus niger*.

		Candida albicans	*Aspergillus Niger*
Ethanol extract	**100**	**18**	**16**
	50	**14**	**14**
	25	**15**	**16**
	12.5	**14**	**15**
	6.25	**11**	**13**
Chloroform Extract	100	18	16
	50	16	14
	25	15	16
	12.5	14	15
	6.25	11	13
n-butanol extract	100	15	16
	50	11	16
	25	15	15
	12.5	11	13
	6.25	8	10
Ethyl acetate	100	19	19
	50	18	20
	25	17	18
	12.5	15	15
	6.25	13	10
Petroleum ether	100	-	-
	50	-	-
	25	-	-
	12.5	-	-
	6.25	-	-
Water extract	100	-	-
	50	-	-
	25	-	-
	12.5	-	-
	6.25	-	-

Testing of Extracts for Antifungal Activity

Here again petroleum ether and water extract showed negative activity against the standards fungi in use. An increase of inhibition zone is observed when increasing the concentration to 100mg/mL.

Testing the Antiinsecticidal Activity

To assist the antiinseticidal activity of our extracts, we count the mortality of the second larval African melon ladybird beetle during 24, 48 and 72 hours as shown in Tables 4,5 and 6.

Table 4.1. Results of mortality of second larval instars of African melon ladybird beetle in 24 hrs

Concentration %	Results			
	R1	R2	R3	Mean
1.25%	0	0	0	0
2.5%	0	0	0	0
5%	10	10	10	100%
standard	10	10	10	100%
control	0	0	0	0

Table 4.2. Results of mortality of second larval instars of African melon ladybird beetle in 48 hrs

Concentration %	**Results**			
	R1	R2	R3	mean
1.25%	1	1	0	6.6%
2.5%	2	7	4	43.33%
5%	10	10	10	100%
standard	10	10	10	100%
control	0	0	0	0

Table 4.3. Results of mortality of second larval instars of African melon ladybird beetle in 72 hrs

Concentration %	Results			
	R1	R2	R3	Mean
1.25%	2	5	1	26.66%
2.5%	10	10	6	89.55%
5%	10	10	10	100%
standard	10	10	10	100%
control	0	0	0	0

At 24 hrs, only the concentration of 5% of the extract is capable of giving an antiinsecticidal activity comparable to that of the standard, while 1.25 % and 2.5 % gave no mortality at all. At 48 hrs we begin to see a weak mortality with the concentration 2.5 %. At 72 hrs, a strong mortality is already seen with 2.5 % with weak to medium mortality with 1.25%.

Table 4.4. Effect of ethanolic extract on the mortality of second larval instars of African melon ladybird beetle.

Concentration %	**Mortality %**		
	24 hours	48 hours	72 hours
1.25%	0.0 (0.0)	6.66 (12.2)	26.66 (30.0)
2.5%	0.0 (0.0)	43.33 (40.86)	89.55 (76.92)
5%	100 (90)	100 (90)	100 (90)
Cypermetherin 25% at 4ml/L	100 (90)	100 (90)	100 (90)
control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
SE+	10.27	6.60	12.03
C.V%	17.78	60.40	42.83

Conclusion

In this study phytochemical screening of the leaves of plant *solenostemma argel* was performed in order to determine its components which can have important roles in the protective activities hold by the plant. We have detected different constituents such as Alkaloids, Flavonoids, terpenoids, saponins, tannins, steroids, and anthraquinons in the extract and fraction extract. Since *solenostemma argelis* is widely used against different diseases especially infectious, we assisted its antimicrobial activity and insecticidal effect. The crude extract and extract fraction were investigated for their inhibition activity against bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruinosa and Escherichia coli*) fungi (*Aspergillus niger and Candida albicans) a*nd insect second larval of African melon ladybird) and exhibited effectively high growth inhibition and larval mortality respectively.

Solenostemma argel could be used in therapeutic procedures as antibiotic for infectious diseases or antifungal and anti insecticidal in agriculture to maintain and protect agricultural plants. It is rich in organic components and the fact that is natural and widely spread makes it a good and potential candidate for pharmaceutical industries.

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