Mycopath (2018) 16(2): 65-72 **Research Article**

Phytochemical screening of Algerian medicinal plants and their antimicrobial effects

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Abstract

In order to test the importance of using some medicinal plants in indigenous medicine system to treat various diseases, this study examined the antimicrobial effects of aqueous extracts resulted by decoction and infusion of a local plants from Algerian South-west zone: *Atriplex halimus* L. and*Ficus carica*L. The antimicrobial properties were examined by disc-diffusion technique against eight (08) bacterial strains, and one (01) strain of fungus. The minimum inhibitory concentration (MIC) and interaction tests, between the different bacteria, *fungi* and plants extracts were determined by the checkerboard method on Petri dishes. Phytochemical composition analysis allowed for the quantification of total polyphenols, flavonoids and anthocyanins contents of plants aqueous extracts. The decoction of *Atriplex halimus* inhibits the growth of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* species inside inhibition areas with diameters about 13.5 and 14 mm, respectively.The infusion presents an appreciable inhibitory action with an inhibitionzone of 16 mm against *Salmonella typhimurium.* Both two extracts of *Ficus carica*were inactive against all the microbial species tested.
Keywords: Aqueous extracts, Antimicrobial effects, *Atriplex halimus*,*Ficus carica*, Medicinal plants.

Introduction

Natural compounds from plants are attracting more and more attention for their potential uses in the treatment and prevention of infectious diseases. Pharmacopoeia has become more attracted because of both the increasing cost of drug production and the abuse of antibiotics and their inappropriate use that lead to multidrug-resistant bacteria emergence (Traoré et *al.* 2012).

According to the magazine " *The Economist* ", the world market of aromatic and medicinal plants is estimated at about 64 billion dollars with more than 35000 plants that are exploited in the world for cosmetic, chemical, pharmaceutical, therapeutic, herbalist, agro-food, aromatic and culinary purposes. In addition, they are also the basis of processed natural products with high added value such as essential oils, dry extracts, liquids and oleoresins (Hmamouchi,1997; Blasco, 2012)**.** Many aromatic and medicinal plants are also well known to have antibacterial activity against different pathogens through their secondary metabolites. It will be necessary to develop alternative methods, natural and safe, to fight against infections (Vegara *etal.*, 2011; Fratini et *al.*, 2014)**.**

Atriplex halimus belongs to the family *Chenopodiaceae* (Linnaeus, 1758), which has 100 genera and more than 1700 species. It is worldwide distributed, especially in desert and semi-desert areas in soils containing much salt. Among about 14 genera of the *Atripliceae* group, the *Atriplex* genus is the largest with more than 100 species(Dinan *etal.,* 1998; Fuentes Bazan *et al.*, 2012; Lamchouri *et al*., 2012)**.** This species was recorded in the literature to have various uses. It is one of the most preferred forage shrubs for livestock as it could cover its needs for essential nutrients (Haddioui and Baaziz, 2001; Nemat Alla *et al*., 2012). It has traditionally allowed the treatment of certain diseases such as cardiovascular diseases, diabetes and rheumatism. It also serves to regulate bile secretions. The extract of *A. halimus* acts by its antioxidant power in the reduction of high levels of LDL, its alkaline character and allows the manufacture of soap(Said *et al.*, 2008; Martirosyan and Steinberger, 2014).

The genus *Ficus* is a large one, with hundreds of species (Dickson, 1996) like *Ficus carica* which are commonly known as figs. It is one of the unique *Ficus* species widely spread in many countries with edible fruits with high commercial value (Vikas et *al.,* 2011; Irudayaraj *etal.,* 2016). Many *Ficus*species have long been used in folk medicine for the treatment of respiratory diseases, jaundice, diabetes, inflammation and for its hepatoprotective, hypoglycemic, antifungal, antipyretic, antioxidant and antimutagenic properties. It has been also largely used as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and antidysentery drugs (Mousa *etal.,* 1994; Guarrera, 2005; Vikas *etal.,* 2011).

The world-wide interest in the medical contest

to develop new antimicrobial agents has led to a search for new natural sources and, therefore, antimicrobial acticities of *Ficus carica* and *Atriplexhalimus* aqueous extracts were examined.

Materials and Methods

Plant material

The plant material was collected during August-September 2017 from the Saoura region situated on the west of Algeria under latitude 31°,37'0 North and longitude: -2°,13'0 West. This place located among Algerian arid and semi-arid areas (mean annual rainfall < 100 mm). Aerial parts of *A. halimus* and the fruits of *F. carica* were air-dried at room temperature for 30 days and crushed.

Preparation of aqueous extracts

To prepare decoction extracts, the powdered aerial parts of *A. halimus* (50 g) was extracted with boiling water (500 mL) for 15 min(Kraft and Hobbs, 2004). The next step, was the infusion of (50 g) of dried aerial parts of the selected aromatic plant with 500 mL of initially boiling water for 15 minutes in order to enable the diffusion of active substances to the water (Sophie and Ehrhart, 2003). The extracts were then completely filtered, concentrated in rotary evaporator and stored at 4 °C until they were used for antimicrobial assay (no longer than 6 months).

Microorganisms

For the antimicrobial test, bacterial and fungal strains of international collections were used and were provided by the Laboratory of Biotoxicology Pharmacognosy and Biological Valorization of Plants, University Tahar Moulay, Saida, Algeria, distributed as follows:Gram positive bacteria namely*Enterococcus faecalis, Staphylococcus aureus;* Gram negative bacteriaincluding*Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonia, Escherichia coli, Enterobacter cloacae, Citrobacter freundii*; and the fungus*Candidaalbicans*.

Determination of the dry matter content

The content of water (relative humidity) corresponds to the loss of mass undergone by the sample after heating in an oven at 103 ± 2 °C until constant weight, the mass reached when during the drying operations the difference between two successive weightings of the sample cooled at 4 h interval does not exceed 0.1% compared to the last determined mass. Three measurements were made. The content of water and volatiles is expressed as a mass percentage (Marianne, 2013).

$$\% \text{ Water} = \frac{m1 - m2}{} * 100 = H$$

$$\% \text{ Dry matter} = 100 - H = \frac{m2 - m0}{m1 - m0} * 100$$

the cup and of the test sample before heating in g.
m_2: the mass of the dish and of the residue after heating to constant weight in g.

Phytochemical screening of extracts

For determination of total polyphenols content (TPC),first, the extraction yields (EY) were calculated and then according to the Folin-Ciocalteu method, 200 µL of extract was mixed with 1 mL of Folin (10 times diluted). After 4 min, 800 µL of sodium carbonate (75 mg mL^{-1}) was added. The incubation was carried out for 2 h at ambient temperature and the absorbance was measured at λ = 760 nm. The concentrations was expressed in gallic acid equivalent (mg GAE g^{-1}) (Ćujić *et al.*, 2016).

Total flavonoids were calculated according to the method of aluminum chloride, in which 1 mL of 2% methanolic solution of $AlCl_3$ was added to 1 mL of extract, then the absorbance was measured at 430 nm, after 10 minutes of incubation. Concentrations expressed in quercetin equivalent (mg QE g^{-1}) (Ghedadba *etal.*,2014).

In order to determine the total anthocyanins content, the pH differential method was used, which consists in measuring the absorbance of the mixture at two wavelengths: 510 and 700 nm by two buffer systems at pH 1.0 and at pH 4.5. Concentrations were expressed as cyanidin-3-glucoside equivalent (mg C3G g^{-1}) (Lako *et al.*, 2007; Ercisli *et al.*, 2012).

$$A = (A510\text{-}A700)_{pH1,0} - (A510\text{-}A700)_{pH4,5}$$

$$TAC = [(A \times MW \times DF) / MA] \times 100.$$

Where, A: absorbance; MW: molar mass; DF: dilution factor; MA: molar absorption. All these measurements were carried out in triplicate.

Antimicrobial properties assays

Antimicrobialactivities of plants crude extracts resulted by decoction and infusion of aerial parts were assessed using disc-diffusion method (Murray *etal.*, 2016). The aromatogram is a technique similar to the antibiogram, a routine test for bacteriological laboratories to test the susceptibility of strains to antibiotics. In this technique, the loaded discs were placed on the surface of the solidified medium (Mueller Hinton Agar) already inoculated with the bacterium tested. The next step was the incubation for 24 h at 37 ºC for bacteria and 48 h at 27 °C for the fungus. A negative control was prepared using respective solvent. Antimicrobial activity was assessed by measuring the inhibition zone diameter around the disc. All the tests were performed in triplicate.

The antimicrobial capacitiesof *A. halimus* and *F. carica*extracts were compared with those of ampicillin (AP) and rifampicin (RF) used as positive controls. Empty standard antibiotic disks were used as a negative control. Minimum inhibitory

concentrations (MICs) are defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *invitro* activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints (Andrews, 2001; El Amri *et al.*, 2014).

Statistical analysis

Statistical studies of all tests results were performed via one-way analysis of variance (ANOVA) in order to measure the correlation between the composition of extracts and their antimicrobial action. The significance level for the analyses was set to $P< 0.05$.

Results and Discussion

The dry matter composition summarized in Table 1,Fig. 1 and 2showed the levels of total polyphenols, total flavonoids and total anthocyanins (means±standard deviation). The Folin-Ciocalteu reagent, used in the determination of polyphenols, consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolibic acid ($H_3PMo_{12}O_{40}$). Its reduction by phenolic compounds, in alkaline condition with sodium carbonate gives rise to a blue-colored mixture of tungsten oxides (W_8O_{23}) and molybden (Mo_8O_{23}). The intensity of blue coloration detected at at 760 nm, the maximum of absorption, reflects the phenolic compounds amount in the sample expressed in milligrams equivalent of gallic acid (or catechin) per gram of dry weight (Georgé et *al.*, 2005).

The flavonoid content of extracts is measured by the aluminum chloride test. In fact, the flavonoids possess a free hydroxyl group in the position 5 capable of giving, in the presence of aluminum chloride, a yellowish "aluminum-flavonoid" complex following the chelation of Al^{+3} ion. The yellow coloration produced, detected at 510 nm, is proportional to the quantity of flavonoids present in the extract (Basli et *al.*, 2012), (Li et *al.*, 2015). The pH differential method allows the determination of anthocyanins in a sample. It is based on the fact that anthocyanins undergoing structural changes reversible by pH change, expressed by different absorption spectra. The colored form (Oxonium) predominates at pH 1.0 and the colorless form (Hemicetal) at pH 4.5 (Tonutare et *al.* 2014).

Both, *A. halimus* extracts decoction and infusion have presented appreciable amounts of total polyphenols of about 87.53±0.33 and 72.16±4.12 mg GAE /g, in the order already mentioned. On the other hand, decoction and infusion of *F. carica* have TPC values of 37.22±4.55 and 31.66±2.32 mg GAE /g, respectively. An important TFC content for *F. carica* was noted of 28.08±1.66 mg QE/g. The variations observed in the phenolic profiles of different samples can be explained by the growth conditions: geographical location, soil type, climate, precipitation, altitude, harvest season, the duration of conversation, diseases that can affect the plant and the treatments carried out, which directly interfere with the content of chemical constituents and consequently their therapeutic effects (Özgüven & Tansi, 1998), (Park & Cha, 2003), (Naghdi Badi et *al.*, 2004), (Ebrahimzadeh et *al.*, 2008). The average of dry matter yields for *T. vulgaris* which grew for 120 days varied, depending on the type of light used and the soil water amount (SWA), between 7,00g (SWA = 50%) and 18.20g / plant (SWA = 90%) under natural light, and from 19.70g (SWA = 50%) to 50.30g/ plant (SWA = 90%) under additional light. This content has gradually increased from 15% to 29%,whereas the variants of plants under natural light had lower dry matter contents than those grown under an additional light(Letchamo et *al.*, 1995a).Aqueous extract of *Atriplex halimus* L. was found to have 1,385 mg/g flavonoid and the high result of phenolic content in this plant was found in butanolic extract in leaves and stems 1.2 mg/g (Smara et *al.*, 2016). Extraction yields obtained for the infusion and decoction extracts were 4.76 and 6.28% for *A. halimus*, 3.91and 2.65% for *F. carica.*

The results were in agreement with those of (Macfoy & Cline,1990), in which percent yields (w/w) for the cold and hot fresh leaf aqueous extracts were 3.4 and 1.6% for *F. exasperata.* However, percent yields (w/w) for the methanol dry leaf extract was 8.3% (Macfoy & Cline,1990). Although, water is almost universally the solvent used to extract activity, it has been cited that solubility ability of different phytochemical compounds varied depending to the solvent used. (Cowan, 1999). It has been observed that acetone is a very useful extractant because it dissolves many hydrophylic and lipophylic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used.

After acetone, he ranked other solvents in the order a mixture of Methanol:Chloroform:Water, methanol, ethanol and water (Eloff, 1998). In this study, methylene chloride and butanol extracts of *A. halimus*also exhibited a positive effect against *E. coli*, *S. typhimurium* and *S. aureus* with inhibition diameter (mm) of 12.5,11.75 and 11.5 mm for the first and 10, 9.75 and 9.5 mm for the second, respectively. On the other hand the ethyl acetate extract was ineffective against these bacteria strains (Gattouche et *al.*, 2018).Sensitivity to different oils is classified according to the diameter of the inhibition zones: “not sensitive” the diameter is less than 8 mm; “sensitive” for a diameter between 9-14 mm; “very sensitive” between 15-19 mm and “extremely sensitive” if the diameter is more than 20 mm.The *A. halimus* decoction extract prevents the growth of the two pathogen strains *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition

areas diameters about 13.5 and 14 mm, respectively where MICs were 141 µg/mL. However, this extract appears ineffective against almost all the rest of the microorganisms tested.The infusion presents an acceptable inhibitory effect of 16 mm versus *Salmonella typhimurium.* All extracts of *Ficus carica* were ineffective against all the bacteria strains tested. The MIC noted for it was 141 µg/mL. None of all aqueous extracts showed an activity against the *Fungus, Candida albican.* These estimates have been promising, because the tested extracts have been able to inhibit the following bacteria: *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococcus aureus* classified as highly pathogenic germs.

Through the study realized by Messaoudi, 2016, extraction yields of infusion and decoction extracts were 4.79 and 6.34 % for *A. halimus*,TPC levels were 88.23 and 84.64 mg GAE /g. Except *S. aureus,A. halimus* infusion the growth of all other bacteria tested with maximum inhibition zone of 14 and 13mm against *P. aeruginosa* and *S. typhimurium.* Thestrains *S. typhimurium, E. coli* and *K. pneumoniae* were resistant to decoction (Messaoudi, 2016). Plants synthesize aromatic substances with an almost limitless ability, most of which are secondary metabolites such as flavonoids and phenolic compounds. These constituents serve as plant defense mechanisms against predation by herbivores, insects, and microorganisms (Cowan, 1999). Plants have always been excellent sources of drugs and many of the currently available drugs have been derived directly or indirectly from them (Surya et *al.,* 2014).It was realized early that the genus *Ficus* was promising as a likely source (Abu-Mustafa et *al.*, 1964). The main antimicrobial effect is related to Flavonoids as they can interact with the bacterial cell wall and lipophilic flavonoids could disrupt it (El-Aasr et *al.,* 2016). All bioactive substances of plants have been shown to be responsible for the antimicrobial capacities observed by these extracts. Aqueous extracts with remarkable antibacterial activity have high levels of polyphenols and flavonoids.

It is well known that the glycosylated flavonoids, unlike aglycones, are hydrosoluble, it is therefore possible that they are responsible for this activity (Marston & Hostettmann, 2006).

Clearly, reducing the size of the bacterial screen would have resulted not only in the failure to retrieve valuable information but, more importantly, in reporting misleading negative data. However, the number of organisms used in a screening and must often be restricted due to resource limitations. In classifying the activity of the antibiotic extracts as Gram-positive or Gram-negative, it would generally be expected that a much greater number would be active against Gram-positive organisms than against Gram-negative. (McCutcheon *et al.*, 1992).

The statistical analyses revealed that no significant differences exist between water extracts ($p < 0.05$). The results for *A. halimus*are in agreement with previous reports showing biological activity of aqueous extracts, such as decoctions (Martins et *al.,* 2015). The concentration of an extract may influences its effect, the study of about antibacterial activity of methanol extract (6 mg/ml) of *A. halimus* was tested against pathogenic bacteria. While *E. coli* and *S. enterica were* resistant to it, zone of inhibition (mm) were 8, 5, 1, 20 mm against *K. pneumonia, S. aureus* 72, *S. aureus* 132 and *S. aureus* 224. Using (23 mg/ml) of *A. halimus*methanol extract, obtained zone of inhibition (mm) were 9, 10, 3, 25 mm against *K. pneumonia, S. aureus* 72, *S. aureus* 132 and *S. aureus* 224. *E. coli* and *S. enterica* were also resistant to it (Abdel Rahman *et al.*,2011).

Each of the three extracts cold aqueous, hot aqueous and methanol of *Ficus exasperata* were inactive against both bacteria species and *E. coli S. aureus* (Macfoy & Cline,1990). The acetone extracts of the leaves of *F. carica* L. and *F. erecta* showed antibacterial activity against *Staphylococcus* species, but were not effective against *Pseudomonas syringae.* The extract of *F. carica* possessed antifungal activity against *Fusarium solani, F. lateritium, F. roseum, Daporuthe nonurai* and *Bipolaris leersiae* (Mousa et *al.,* 1994). Mousa et *al.,* 1994, tested the bioactivity of four (04) Egyptian Ficus species extracted under ultrasonication using chloroform as solvent. None of the four extracts showed any antifungal activity. On the other hand, they displayed inhibition ability against both Gram negative and Gram-positive Bacteria. Inhibition zones produced were as follow: * *Ficus sycomorus* L., *E. coli* (17-20 mm), *KIebsiella pneumonia* (16-19 mm), *Pseudomonas aeruginosa* (16-19 mm), *S. aureus* (16-19 mm). * *F. benjamina* L., *E. coli* (17-20 mm), *Pseudomonas aeruginosa* (16-19 mm), *S. aureus* (17-20 mm) * *F. religiosa* L., *E. coli* (-), *KIebsiella pneumonia* (16-19 mm), *Pseudomonas aeruginosa* (-), *S. aureus* (-) * *F. bengalensis* L. *E. coli* (16-19 mm), *KIebsiella pneumonia* (16-19 mm), *Pseudomonas aeruginosa* (16-19 mm), *S. aureus* (16-19 mm).

There is a direct correlation between the effects of molecules extracted from medicinal plants and their chemical composition. In bacteria, these compounds act by inhibiting bacterial growth, sporulation and toxin synthesis; In the fungi, their action, would be limiting the germination of spores, the formation of mycelium and the production of toxins (Caillet & Lacroix, 2007) The antibacterial activity of a given extract is the consequence of the presence in this extract of the molecules which can act alone or by synergy between them on one or more bacteria (Sarker et *al.,* 2005). The activity of a plant is commonly related to the presence of the major active principle it contains. The term "*totum*" refers to all the constituents of the plant that

are supposed to be active, acting in synergy and by complementarity to modulate, moderate or reinforce the activity of the drug (Mansour-Djaalab et *al.*, 2014-15).

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Conclusion

The recorded results indicated that the two extracts obtained by decoction and infusion of *A. halimus* showed a powerful antibacterial activity towards the majority of the bacteria examined. These observations will be the basis for future studies concerning the capacity of certain extracts of medicinal and aromatic plants to confer biological and curative effects. Other outstanding studies about these plants will be needed to isolate the bioactive constituents to use them for pharmaceutical purposes.

Table 1: The dry matter composition (DM) of *A. halimus* and *F. carica*.

Studied plants	DM (%)	DM (g)
A.halimus	57.5±1.697	2.875±0.084
F. carica	36.4±4.323	1.820±2.039

Table 2: Inhibition zones diameters (mm) of microbial growth induced by aqueous extracts of *A. halimus* via the disc diffusion method.

Bacterial Strains Tested	Inhibition zone diameter (mm)			
	A. halimus		Positive control	
	Infusion extract	**Decoction extract**	**AMP**	**RF**
Citrobacter freundii	-	-	-	-
Escherichia coli	07	-	9	7
Enterobacter cloacae	-	10	-	-
Salmonella typhimurium	16	-	12	-
Klebsiella pneumonia	13	-	15.5	-
Pseudomonas aeruginosa	09	14	-	-
Staphylococcus aureus	11	13.5	14	9
Enterococcus faecalis	-	09	12	8
Candidaalbicans	-	-	-	-

(-): Absence of activity; Negative controls did not show any activity. RF: rifampicin; AMP: ampicillin.

Table 3. Minimal inhibitory concentrations of *Atriplex halimus* extracts.

Bacterial Strains Tested	MIC (µg mL^{-1})	
	Infusion extract	**Decoction extract**
Citrobacter freundii	-	-
Escherichia coli	230	-
Enterobacter cloacae	-	92
Salmonella typhimurium	141	-
Klebsiella pneumonia	230	-
Pseudomonas aeruginosa	141	78
Staphylococcus aureus	141	256
Enterococcus faecalis	-	92
Candidaalbicans	-	-

Note: MIC - Minimal Inhibitory Concentration; (-): Absence of activity; Negative controls did not show any activity.

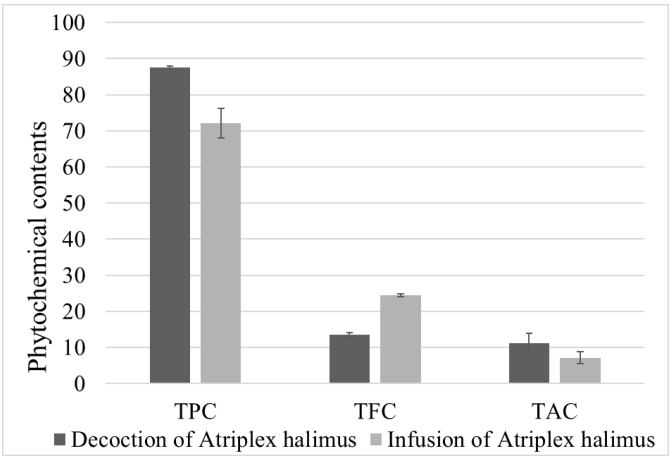

Fig. 1: Amounts oftotal polyphenols, flavonoids and anthocyanins of *A. halimus* aqueous extracts.
TPC: Total Polyphenols Content(mgGAEg^{-1});**TFC:** Total flavonoids content(mgQE g^{-1}); **TAC:** Total anthocyanins content **(**mgC3G g^{-1}**)**.

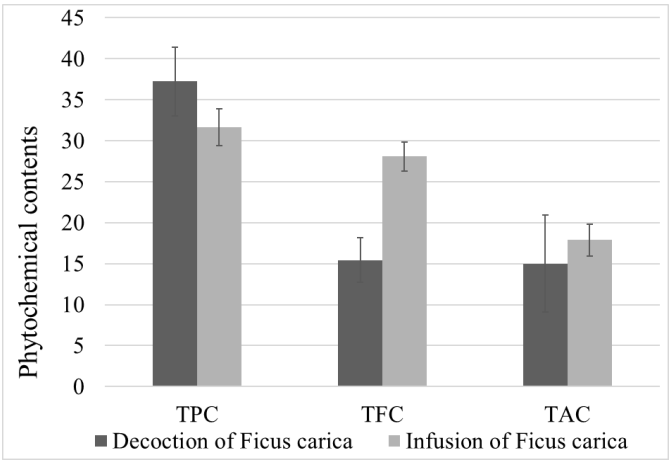

Fig. 2. Amounts oftotal polyphenols, flavonoids and anthocyanins of *F. carica* aqueous **TPC:** Total Polyphenols Content(mgGAEg^{-1});**TFC:** Total flavonoids content(mgQE g^{-1}); **TAC:** Total anthocyanins content **(**mgC3G g^{-1}**)**.

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