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Evaluations of Antimicrobial Activity of Aqueous, Ethanolic Extracts of *Peganumharmala* L. Against Pathogenic Bacteria Isolated From Different Sources

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ABSTRACT

Background: The aim of current study to Isolation and identification of causal agent aerobic bacteria which Cause a variety of diseases in cows and Evolution of antimicrobial activity of aqueous, ethanolic extracts of against pathogenic bacteria isolate.

Material and Methods: A total 100 samples (Milk, Feaces, bone fracture, ocular discharge and mouth inflammation) were taken from Various diseases in cows. The results showed to the type and ratio of bacterial isolated were *Staphylococcus aureus* 35 (31.8 %), *E coil* 25(22.7%), *proteus mirabilis* 20 (18.8%), *Klebsilla puenmonia* 15 (13.6 %) *staphylococcus epidermidis* 10 (9.1%), and Micrococcus .Spp 5(4.5%). The antibiotic sensitivity results showed the Ciprofloxacin is the drugs of prefer use for treatment to a variety of diseases in cows.

Results: The results ethanolic extracts showed capability of *Peganumharmala* L to inhibition growth pathogenic bacteria. The greatest inhibition zone reached 55 mm to ward *Klebsiella pneumonia* at concentration 200%. while the range inhibition zone (45, 4, 35, 30, 27, 25 and 15) mm at concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively. and lowest inhibition zone zero mm toward *proteusmarbilis at* concentration 200%. while the range inhibition zone (30, 22, 24, 24, 20, 17 and 16) mm at concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively.

While the results aqueous extract showed capacity of *Peganumharmala* L to inhibition growth pathogenic bacteria. The maximum inhibition zone reacher 50 mm to ward *Klebsiella pneumonia* at concentration 200%. And the range inhibition zone (49, 35, 30, zero, zero and zero) mm at concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively while minimum inhibition zone zero mm *were E. coil at* concentration (200%, 100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively.

Keywords: Evaluations, antimicrobial, activity, aqueous, ethanolic extracts, *Peganumharmala* L., pathogenic,Various diseases, cows.

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INTRODUCTION

The growing interest and the use of therapeutic plants in scientific do research are intensively aimed at replace medicines Chemical reaction with serious side effect on the body and vital organs such as liver and kidney with plant extracts and with minimal harm, but despite these studies, the use of medicinal plants is random by the civic with the aim of medication Without taking into explanation its toxic effect, leading to serious poisoning that may lead to death(1).

Nowadays, bacterial infection especially those cause by multi-drug resistant (MDR) bacteria have become one of the largechallenge for recent healthcare. Therefore, discover new antibacterial compound with improved activity is essential. Majority of scientists identify multi-drug resistance (MDR) as ″resistance to at least 3 classes of antimicrobial agents. Since the original time, the idea of discovery healing power in plants has been noteworthy. Plants create a vast array of secondary metabolites that in many cases, these substance act as safety mechanisms against predation by herbivores, microorganisms and insects; also similar substance can be formed by plants as a part of their normal growth and expansion or in response to stress(2).

The herb, *Peganumharmala* is consider a flower herbal plant, go to the Zygophylaceae family It is characterize by broad geographical extend. The human is expose by the random use of the plant to poison, and symptoms are the fatigue and weakness and severe tension and trouble in the movement of voluntary muscles and increase the discharge of saliva and nausea and diarrhea, and often the symptoms resulting from the injury of the nervous system are prevailing, as noted Increase, respiratory rate, temperature alter and failure of appetite(3).

There are a variety ofinformation that *P. harmala* had unlike pharmacological activities including naturalresult, anti-tumor effect, insecticidal effect, caving malaria, anti-leishmanial, anti-spasmodic, anti-histaminic, vasorelaxant effect, wound therapeutic, anti-oxidant activity, leukemic healing, hypoglycemic effect, immuno-modulator properties, analgesic and anti-inflammatory property. Also, it has been report that this plant had antibacterial, antifungal and antiviral achieve(4).

Peganumharmala is a loaded source of alkaloids Mainly strong in seeds and less in β-Carboline roots mainly to alkaloids on *Peganumharmala*. The pharmacological effect of alkaloids on the physiological stagediffer between temperature decline and psychosocial alter; thus a status of hallucination or may work; as sedatives, inhibition of enzymes such as MAO. and used as an antibacterial agent, treatment of asthma, jaundice, lumbago, and several other humanailments. *Peganumharmala* is usually used as a drinking herb to reduce pain, Menstrual pain and abortion, as well as several skin diseases (5).

In some study, antibacterial action of *P. harmala* was established. According to these research, *P. harmala*extract can inhibit plank tonic form of several bacteria such as *Streptococcus pyogenes, Salmonella typhimurium*, *Pseudomonas aeruginosa, Escherichia coli,Staphylococcus aureus, Staphylococcusepidermidis, Klebsiellapneumoniae*(6).

MATERIALS AND METHODS

Collection and storage of *Peganumharmala* L. seeds

The seeds of *Peganumharmala* L. for the determination of antimicrobial activity were collected from a local market of tikrit .They were grinded with the assist of clean grinder and mortar and were packed in polythene bags for further extractions. Then they were store in a dry place in microbiology laboratory.

Swab samples

Collected 100 samples (Milk, Feaces ,bone fracture, ocular discharge and mouth inflammation) were taken from different sources. by sterile cotton swabs and sterile tubes . Transported to laboratory as soon as possible in sterile Brain heart infusion broth that incubated at 37 C for at least 24-28 hours to increasing chances of isolation described by[7].

Isolation and Identification of Microorganisms

All Samples were cultured into Brain heart infusion broth and then onto MaCconkey agar and mannitol salt agar. All culture plates were incubated for overnight at 37C° described by[8].

Biochemical test

1-Catalaseand coagulase test

Catalase and coagulase tests use distinguish isolated bacterial consistent with method described by[9].

2- Oxidase Test

Cytochrome oxidase is the finishing enzyme in the electron transport chain of bacterial cell respiration. It oxidizes cytochrome C, the electron transport particle and decrease oxygen to shape water. If oxidase was present in the colony, it would oxidize the reagent and the colony on the filter paper would rotate dark blue within one minute. A negative oxidase test resulted in no color alter of the smear (Cowan and Steel (1993)[8].

3-Triple sugar iron gar

Determine sugar fomenters and also the bacteria which create H2S. The isolate bacteria were growth over the surface of the slants and bottom following incubate for 24 hr at 37°C[8].

4-Urease test

Certain bacteria have enzymes called ureases which are able of hydrolyzing urea to yield alkaline ammonia (NH4). The tubes were inoculate with the samples in subject and allowable to protect at 37oC for 24 hours, at which point tests were initially examine. Pinkish or red tubes were interpret as positive. yellow tubes were negative[9].

5- Indole, MR and VP tests

Distinguish the isolation bacterial described by (Cowan and Steel(1993)[8].

6-Citrate utilization test

Citrate test is used to recognize if an organism is able of utilize citrate as a sole carbon source or not. Citrate medium contain sodium citrate as the only source of carbon and ammonium phosphate as the only source of nitrogen .bacteria which have citrate-permease can transfer citrate in to the cell and create pyruvate and show a color change from green to blue. incubation[10].

7-Fermentation test

This test used to differentiate between types of the bacterial which capability of ferment a wide group from sugars and others ferment only a few described by (Ryan and Ray (2004)[11].

Identification using Vitek® compact 2 system

recognition has been done by automatic method (Vitek® 2 Compact, Biomérieux, France) . Gram positive and negative bacteria were classify using Gram positive and Gram negative ID card respectively (Biomérieux, France[12,13].

Antimicrobial sensitivity test

In vitroantibiotic sensitivity test (Kirby-Baur disc diffusion) method was accepted out in order to recognize the most useful drugs for treatment in the study area. A loop full of colony from the growth of isolate was move to the nutrient broth in tubes and incubated at 37°C for 5 h. Mueller-Hinton agar which was used as plating medium was inoculate with broth (bacterial suspension) by using cotton swab. Then antibiotic impregnate paper disc were relate and pushed onto the plate with forceps. Plates were incubated at 37°C for 18 h. The diameters of zones of growth reserve were calculated in millimeter and read as sensitive, intermediate and resistant to unlike antibiotics[14].

Preparation of*Peganumharmala* L extracts

Two extracts of *Peganumharmala* L were made in ethanol (95%) and distilled water. A 50 ml falcon tube was taken and 160 ml of solvent was added in it. Then 40 g of the powdered *Peganumharmala* was soaked in 160 ml of the solvent. Then it was rotate on the shaking incubator at 150 rpm for 24 h. Then after 24 h, the extract was filtered by filter paper and centrifuged at 3500 rpm for 15 minutes at 4C°. After the centrifugation, the extract was filtered through muslin cloth. The capsule was discarded and the supernatant was centrifuged again until the extract made was 100 % clean and putting in oven at temperature (40-50c°). Then the extract was store at 4C° for future use[15].

Qualitative phytochemical screening in *Peganumharmala* L extracts

1-Detection for flavonoids

The stock solution (1 mL) was taken in a test tube and added a small amount of drop of dilute NaOH solution. An severe yellow color was show in the test tube. It becomecolorless when on adding of a few drop of thin acid that show the presence of flavonoids[16].

2-Detectionfor alkaloids:-

Mayer's reagent was used to decide the occurrence of alkaloids in the ethanolic extract of *Peganumharmala*. To create the Mayer's reagent, 1.36 g of mercuric chloride and 5

g of potassium iodide were added to 100 ml of distilled water in a glass. Then 5 ml of the ethanolic extract of *Peganumharmala* Lwas added in a test tube. 2 drops of the Mayer's reagent was additional to the test tube. The result was noted[16].

3-Detectionfor tannins

FeCl3 test was used to ensure the presence of tannins in the water extract of *Peganumharmala* L To do the test, 5 ml of the boiled aqueous extract of *Peganumharmala* L was added in a test tube. 2 drops of 5 % FeCl3 was additional to the test tube. The result was noted[16].

4-Detectionfor saponins

A distilled water test was used to find the presence of saponins in aqueous extract. 3 ml of the boiled and filtered *Peganumharmala* L extract was added in test tube. Then 3 ml of the distilled water was put in the tube. The solution was agitated for 5 min. After the shaking of 5 min, the reaction was noted. [16].

5-Detection of glycosides (Benedict test)

137 g of sodium citrate and 100 g of Na2CO3.H2O were dissolved in 800 ml of distilled water, the combination were filtrate and added 17.3 g cupric sulfate was dissolved in 100 ml of distilled water and added to the previous filtrate then volume complete to 1000 ml with distilled water.2ml of the prepared indicator was added to a test tube contain 1 ml of plant extract and placed in water bath for 5 minute at 50 C^0 and left to cool, Red impulsive will appear if glycosides are present.[16].

Agar Well Diffusion Method to Evaluate the Antimicrobial Activity

The brain heart Agar was melt on flame and poured in petri plate. After solidification of agar in plate, 100 ml of the bacterial stock solution was allowed to spread in the agar plate using sterile spreader. After a wait of 10 min, a sterilized cork borer was taken and with its help, a well was made in the center of the plate. It was filled with 40 ul of the extract. It was then sited in the incubator for a day at 37°C After 24 h, with the help of scale; the inhibition zone was firm in mm. All of the above steps were carry out in Laminar Flow Hood. The zones of inhibition were determined for all of the two extracts of *Peganumharmala* L against all of the ten bacteria in this way. [17].

RESULTS

Bacterial isolation result

Studies 100 samples look her clinical signs which Isolated different source. isolated six types of bacterial includes in the table (1).

Table1: Causative agent isolated from different source

Bacterial isolate	number	%
Staphylococcus aureus	35	31.8%
E. coli	25	27.7 %
proteus mirabilis	20	18.8%
Klebsiella pneumonia	19	9.2%
Staphylococcus epidermedis.	10	9.1%
Micrococcus Spp	5	4.5%

Biochemical test

Bacterial identification was done by biochemical test (Table 2).

Table 2: Biochemical test results of bacterial isolated

Sorbitol	sucrose	glucose	Citrate utilization test	Vogesproskours	Methyl red	Indole	Catalase test	Oxidase test	Biochemical test / Bacterial isolated
+	+	+	-	-	+	+	-	-	*E. coli*
-	-	-	-	-	+	-	+	+	*Micrococcus Spp*
-	+	+	+	-	-	-	+	-	*Staphylococcus aureus.*
-	-	-	+	+	-	-	+	-	*Klebsiella pneumonia*
-	+	+	+	+	-	-	+	-	*Staphylococcus epidermedis*
-	-	+	+	-	+	-	+	-	*Proteus mirabilis*

Antibiotic Susceptibility Test

Study sensitivity bacterial isolated on the way to Antibiotic disc are chloramphenicol, Erythromycin, Tetracycline, Gentamycin, Sulfatrimethoprime, Ciprofloxacin, Aztreonam, Ceftriaxone, Ampicillin and Nalidixic Acid Table (3) and picture (2).

Table 3: Antibiotic Susceptibility Test on bacterial isolates

Antibiotic										
Isolate bacteria	Ciprofloxacin	Ceftriaxone	Gentamycin	Ampicillin	Aztreonam	Nalidixic Acid	chloramphenicol	Erythromycin	Sulfatrimethoprime	Tetracycline
Staphylococcus aureus.	S	S	S	R	S	M	R	R	S	R
Klebsiella pneumonia	S	S	S	R	S	M	R	R	S	R
E.coil	S	M	R	R	R	R	R	R	R	R
Micrococcus Spp	S	M	R	R	R	R	R	R	R	R
Staphylococcus epidermedis	S	M	R	R	R	R	R	R	R	R
proteus mirabilis	S	M	R	R	R	R	R	R	R	R

Sensitive (S), Moderate (M) and Resistance(R)

Active substance in *Peganumharmala* L extracts

Result in table 4 and picture 3 explain chemical chief detect compound and active groups in *Peganumharmala* L extracts.

Table 4: Compounds and active groups in *Peganumharmala* L extracts

Active compound	Ph indictor	Detect guide	Nigella sativa extracts
Flavonoids	NaOH+HCL	Yellow color	+
Alkaloids	Myers test	red – brown precipitate	+
Tannins	ferric chloride (5%)	blue-green	+
Steroids	Chloroform+sulphuric acid	yellow with green fluorescence	+
Saponins	plant extract shacked	formation of foam indicating	+
Glycosides	Benedict test	Red precipitate	+

Evaluation of the antimicrobial activity of aqueous and ethanolic extracts of *Peganumharmala* L against bacterial isolates

The results ethanolic extracts showed capability of *Peganumharmala* L to inhibition growth pathogenic bacteria. The highest inhibition zone reached 55 mm to ward *Klebsiella pneumonia* at concentration 200%. while the range inhibition zone (45, 4, 35, 30, 27, 25 and 15) mm at concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively. and smallest inhibition zone zero mm toward *proteusmarbilis at* concentration 200%.

while the range inhibition zone (30, 22, 24, 24, 20, 17 and 16) mm at concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively. Table (5)

Table 5: Antibacterial activity of ethanolic extracts of *Peganumharmala* L against bacterial isolates

	Bacterial isolates	ethanolic extracts of *Peganumharmala* L. Inhibition zone (mm)							
		200%	100%	50%	25%	12.5%	6.25%	3.25%	1.5%
1	*Klebsiella pneumonia*	55	45	40	35	30	27	25	15
2	*Staphylococcus aureus.*	45	35	25	24	19	18	zero	zero
3	*proteus mirabilis*	35	30	22	24	24	20	17	16
4	*E.coil*	45	35	30	25	20	zero	zero	zero

While the results aqueous extract showed ability of *Peganumharmala* L to inhibition growth pathogenic bacteria. The maximum inhibition zone reacher 50 mm to ward *Klebsiella pneumonia* at concentration200%.and the range inhibition zone (49, 35, 30, zero, zero and zero) mm at concentration (100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively while minimum inhibition zone zero mm *were E.coil at* concentration (200%, 100%, 50%, 25%, 12.5%, 6.25%, 3.25% and 1.5%) respectively. Table (6).

Table 6: Antimicrobial activity of aqueous extracts of *Peganumharmala* L against bacterial isolates

	Bacterial isolates	Ethanolic extracts of *Peganumharmala* L. Inhibition zone (mm)							
		200%	100%	50%	25%	12.5%	6.25%	3.25%	1.5
1	*Klebsiella pneumonia*	50	49	35	30	zero	zero	zero	zero
2	*Staphylococcus aureus.*	40	35	34	zero	zero	zero	zero	Zero
3	*proteus mirabilis*	35	30	29	zero	zero	zero	zero	Zero
4	*E.coil*	40	30	20	zero	zero	zero	zero	Zero

DISCUSSION

all species has particularresult and causesymptom in people who are infected. Some, if not most people who are contaminated with a pathogenic bacteria do not containsymptom. Immuno-compromised persons are additional susceptible to pathogenic bacteria, Some pathogenic bacteria cause disease below certain condition, such as entrance through the skin via a incise, during sexual action or through a compromise immune job[18]. so that the research was aimed to isolatingthe aerobic microbes causing health troubles and characterizing them microscopically ,biochemically and testing their sensitivity to antibiotics used and Evolution of antimicrobial activity of aqueous ,ethanolic extracts of *Peganumharmala L*against pathogenic bacteria isolates.

The results Showed for the 100 samples (Feaces ,bone fracture, ocular discharge and mouth inflammation) that bacteria isolation ratio was 100% and the total bacterial isolates (114) isolation for 6 different genera of bacteria. The results of our study agreed with[19]. where they tested 60 differ sources samples out of which 40% *Staphylococcus*, 16 % *Streptococcus*, 20% *Escherichia coli*, 10% *Klebsiella* and *proteus* organisms were isolated .

On the other hand study showed that Tow unlike types of bacteria were isolated from a total of (30) faecal samples collected from diarrhea of which (20) isolated for *E. coli* and 10 isolated were *staphylococcus aureus*. The results of our study agreed with[20]. This finding Out of (105) faecal samples, 45 (42.85%) samples were belong to for *E. coli*, 22 (20.95%) samples were positive for *Salmonella spp*., 16 (15.23%) samples were positive for *Staphylococcus spp*. It is reported that more than one predisposing factors such as environmental and organization factors (housing, climate), difference nutrition, immune condition of the calves etc. might help in the manufacture of calf diarrhoea along with the presence of one or more than one types of bacteria [21].

The negative and positive results of bacterial culture were found in 10 swabs from bovine bones fracture which included 5 isolated to *Staphylococcus aureus* and 5 isolated *proteusmirbilis.* The results of our study agreed with [22]. Bacterial species found in the animals *Staphylococcus aureus* (10%), *proteus spp*. (10%), *Corynebacterium spp*. (10%), *Serratialiquefaciens* (10%) and *Escherichia coli* (10%).

consider the surgical site infection etiology as multi-factorial, the sterilization, preoperative patient training and operating theatre environment must be careful as the main source of this bacterial contamination. Antiseptics used in our put into practice were theoretically believed to be effective. Since providence- -iodine and chlorhexidine have a broad spectrum of antimicrobial action against vegetative bacteria, fungi, viruses, protozoa, and yeasts, they are suitable for remove bacteria from the skin surface at preoperative surgical site .in addition, povidone-iodine surgical scrub is also used expansively in veterinary practice for the pre-operative training of patients and surgeons [23].

Two aerobic isolates were isolated from infection eyes,the most widespread isolates were *Staphylococcus. aureus* and *proteus spp*. The results of our study agreed with[24]. Bacterial species found in the animals were, *Staph. aureus*, *Proteus. Spp, Staph. Spp, Morexella. spp., Corynebacterium. Spp. Streptococci .spp*. *Pseudomonus. Spp. Bacillus. Spp. and Micrococcus. Spp*. in percentage rates 23.09% ,17.94% ,15.38% , 12.82% ,10.25 % ,7.69% ,5.12% ,5.12% and 2.56% respective. The high ratio as these bacteria are piece of the Bacterial environment for humans and animals worldwide its located on the skin and in the introduction nostrils , in the gut of humans and animals, as they are establish in polluted air, water and soil as well as The fact that many of them severely pathogens mainly pathogenic bacteria (*Staph. aureus*) which produce coagulase enzyme, which accounted for 19% in this study[24].

Antimicrobial susceptibility tested establish for ten antibiotics and the sensitivity of bacterial isolates to antibiotics was determined by measure the diameter of theinhibitory area produced about antibiotic disc used in the study and compare with the special table according to (25).

Antibacterial resistance has been observed for several antimicrobial agents. Antimicrobial resistant cow pathogens may result in treatment failure, leading to economic losses, but also be a source of resistant bacteria/genes (including zoonotic bacteria) that may represent a risk to human health[26].

Despite increaseadvance in medical science and the increase of treatment techniques, infectious diseases are still careful as major cause of worldwide deaths [26].Pathogenic microorganisms have different ways to deal with antimicrobial agents such as antibiotics and indiscriminate uses of these compound have led to the development of drug resistance. Drug- resistant bacteria, is easily crossed antibiotic treatment and generate many clinical problems. Even drug sensitive bacteria that are capable to form biofilms, when located in this formation will act in responsein a different way to antibiotics [27].

Resistance of microbial biofilms to antimicrobial agentlead to solid removal of these microbial formation and cause chieftroubles in controlling of pathogenic microorganisms

and treatment of contagious disease. Biofilm produce bacteria cause more than 60% of nosocomial infection. In the modern years some researchers conduct in order to find new antimicrobial compound against bacterial biofilms, in this case medical plants have a special note[27].

The advantages make use of of medical plant for bacterial illness treatment instead of substance antimicrobial agents includes: Many of the medicinal plants due to a mixture of herbal ingredients with other elements, make balancing the biological toxicity and they have fewer side effect. Also easy access, reasonable price, and no bacterial resistant to herbs are other profit of using medical plants [28].

The study shows a better substitute of frequent antibiotic drugs may be folk medicine. *P. harmala* as antitumoral, antibacterial, antifungal, anti-parasitic, anti-nociceptive, anti-inflammation, vaso-relaxant, and anti-spasmodic activities. This plant is also use for diabetes, jaundice, asthma, dermatitis and many other illnesses.[29] Pharmacological property of *P. harmala* are credited to the creation of alkaloids in different part of the herb. The most important alkaloids in *P. harmala* are beta-carbonyl derivations such as harmalin, harmalol, peganine, isopeganine, deoxyisopeganine; as well as quinazolineorigin such as vasicinone, vasicine, and deoxyvasicinone. Most alkaloids of this herb are resulting from the seeds and roots. Harmalin is the best-known alkaloid in several researches that was studied.(30) The ability to intercalate DNA and the resultant frame shift mutation are amongst the etiological factors for antibacterial result of this plant

The results in Table (5 ,6) proved activity alcoholic soluble matter are more strong against tested microorganisms than aqueous soluble substances and Gram-negative bacteria are more sensitive than Gram-positive bacteria. The results of our study decided with(31). indicate that Gram-negative bacteria are more sensitive than Gram-positive bacteria. (32)clarify this event by the fact that Gram-negative bacteria are endowed with a layer of peptidoglycans wedged between plasma membrane and an external layerfinished up of lipopolysaccharids and proteins. These proteins play a part of a barrier against the plant extracts. (33) disagree with this report. They substantiate that each bacterium Gram-positive is different from a bacterium Gram-negative in its arrangement and its job.

CONCLUSION

Based on the results obtain in this study, it may be complete that plant extracts of *Peganumharmala* have a stronger and broader spectrum of antimicrobial activity against a quantity of food borne bacteria and the extracts may be used to see bioactive normalyield that may serve up as basic source for the development of new antimicrobial compound to overcome the trouble of increase resistance to recognized traditional antibiotics.

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CONFLICT OF INTEREST

Nil

SOURCE OF FUNDING

Self

ETHICAL CLEARANCE

None

REFERENCE

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