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ANTI-MICROBIAL ACTIVITY AND FTIR SPECTROSCOPIC ANALYSIS OF *ARGEMONE MEXICANA* L. LEAVES EXTRACTS

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Keywords:

FTIR, *Argemone mexicana* Linn, phytochemical, Mexican poppy, bacterial skin infections.

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ABSTRACT: The Screening of medicinal plants for the new bioactive compounds leads to the development of new anti-microbial drugs that possess lesser cost and greater safety and efficacy. The increasing bacterial resistance exhibited by microorganisms causing skin infections has led to the research on medicinal plants. *Argemone Mexicana* Linn is a weed that shows multiple health benefits. Agar the parts of the plant are traditionally well known for the treatment of skin diseases. The aim of present study is to investigate and compare the anti-microbial activity of aqueous and ethanol extract of leaves of *Argemone mexicana* Linn against skin infection-causing bacteria. Also, Functional groups associated with phytochemical compounds of these plant extracts are well understood from FTIR Spectroscopy. The *Argemone mexican*a Linn leaves extract shows significant anti-microbial activity against all the selected microorganisms for study. The results for anti-microbial activity were recorded in zones of inhibitions. The highest zone of inhibition had shown by the ethanol extract at higher concentration, *i.e.,* 300 mg/ml against all pathogens. The MIC values of ethanol extract were found to be 20 mg/ml and for aqueous extract 25 mg/ml. The results revealed that ethanol extract is more effective than aqueous extract. This study also revealed that the presence of the functional group in medicinally used plant extracts of *Argemone mexicana* Linn revealed 7 numbers of peak values from Aqueous extracts and 12 numbers of the peak for ethanol Extracts respectively.

INTRODUCTION: Antibiotics are one of the most important weapons for the fight against bacterial diseases and are greatly associated with quality of human life. However, over the past few years, these health benefits are declined due to the development of bacterial resistance. Also, these antibiotics produce severe toxic effects; there is an urgent need to introduce new drugs from natural products with the least toxic effects.

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Medicinal Plants contain several secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, phenols, *etc.*, which possess significant anti-microbial properties. Anti-microbial properties of plants are increasingly reported from different parts of the world [1]. *Argemone mexicana* (Linn), belonging to the family Papaveraceae, is commonly known as Mexican poppy or prickly poppy.

The plant is a weed that is commonly found in waste places. The plant is also known to possess anti-malarial, anti-microbial, antibacterial and antifungal activities [2]. The preliminary phytochemical investigation revealed the presence of carbohydrates, flavonoids, alkaloids, tannins and steroids, *etc*.

The seeds are also used as purgative and sedative in the Indian system of medicine, also useful in leprosy in Homeopathy; also, the juice of this plant is used for the treatment of bronchitis and whooping cough [3]. The fresh leaves juice is used externally as a disinfectant for the wounds [4].

The chemical constituents present in the plants will provide valuable information regarding the different functional groups present in the extracts responsible for the therapeutic properties. Various parts of *Argemone mexicana* were widely used in the traditional system of medicines [5] in the Unani system of medicine *Argemone mexicana* Linn used to treat skin diseases [6].

Now day's medicinal plants being the effective source of medicines, either it can be modern or traditional medicines; the advantage of conventional medicines is they are safe, cost-effective, and useful for health [7]. The use of herbal treatment is one of the best ways to treat diseases caused by multi-drug-resistant bacteria. The use of plant extract with its antibacterial properties is a major work done from the last few years. It has become major work in treatment and to prove efficiency the plant extract used as drugs against different types of pathogens.

Phytochemically, the leaves of *Argemone mexicana* Linn contain flavonoids, sterols, tannins, alkaloids, and glycosides [8]. India is one of the world'stop 12 mega biodiversity centers with the presence of over 45000 different plant species. Of these, about 15,000 to 20,000 plants have gold medicinal value. Every day new inspiring information is being added to herbal medicine for the development of new drugs [9, 10]. The herb and its extracts, infusion, decoction, tincture have been used to manage disease or health disorders in medical practices from ancient period [11]. Egwaikhide (2007), in his study, highlighted FTIR analysis of different medicinal plant extracts and confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acid, esters, ethers, amines, aldehyde, and aromatic compounds, *etc*. with significant peaks. In recent years the FTIR findings have played an important role in most pharmaceutical preparations [12, 18].

METHODS:

Collection and Authentication of the Plant: *Argemone Mexicana* Linn healthy leaves were collected from the local market of Latur in July and August, respectively. The plant parts were identified by Dr. R. M. Mullani, department of botany, SRTMU Nanded. Voucher specimens (Voucher ID SRTMUH.S.L.S.BOTH-105) were deposited in the herbarium section of the school of life sciences, SRTMU Nanded.

The plant material is cleaned with water to remove earthy matter and other external pollutants. Then plant parts were shade dried for one month, powdered in a mechanical grinder, and stored in a clean, dry airtight container for further use.

Preparation of Plant Extract: Soxhlet Extraction The air-dried powdered leaves (30g) of *Argemone mexicana* Linn were extracted with ethanol (95% v/v) until complete recovery of all the dissolved plant constituents.

The extract was then concentrated to room temperature and stored in a refrigerator in an airtight container for further use. Similarly, aqueous extracts were obtained by cold maceration process in which powdered drug material was soaked in water for 24 h.

Then the resulting filtrate was concentrated to obtain an aqueous extract of *Argemone mexicana* Linn. The obtained extracts were subjected to systematic preliminary phytochemical screening by qualitative chemical tests [13].

Phytochemical Analysis: Qualitative chemical tests for identifying various phytoconstituents present were carried out on ethanol and water extracts of *Argemone mexicana* Linn constituents as follows [14, 20, 21].

RESULT:

TABLE I: PRELIMINARY PHYTOCHEMICAL SCREENING OF *ARGEMONE MEXICANA* LINNEXTRACTS

Identification test	*Argemonemexicana* Linn Aqueous extract	*Argemonemexicana* Linn ethanol extract
Carbohydrate	-	-
Protein/amino acid	+	+
Fats/ waxes	+	+
Glycoside	-	+
Flavonoids	+	+
Alkaloids	-	+
Terpenes	+	+
Steroids	+	+
Saponins	+	+
Phenolics /Tannins	+	+
Volatile oil	-	-

+ (presence) - (Absence)

Anti-microbial Activity:

Procurement of Microbial Strains: All the strains of microorganism were obtained from NCIM, and *Argemone mexicana* Linn were tested against *Staphylococcus aureus* ATCC NO 2079, *Bacillis substilis* ATCC NO NCIM 2192, Candida albicans ATCC NO NCIM 3557, *Escheria coli* ATCC NO 8739, NCIM 5012.

Preparation of Different Concentration of Extracts: Three different concentrations of aqueous and ethanol extracts of the plants 100 mg/ml, 200 mg/ml, and 300 mg/ml were prepared by using 10% DMSO.

Preparation of Culture Media: Media and standard anti-microbial drugs (discs) were purchased from Hi-Media Laboratories Ltd, India. All the media were prepared in sterile glass petriplates (4 mm thickness) according to the instructions.

Agar Well Diffusion Method: The anti-microbial activity of ethanol and aqueous extracts of *Argemone mexicana* Linn. were determined against different bacteria at three different concentrations of each extract by the agar well diffusion method. Agar well diffusion method was measured the zone of inhibition to know the anti-microbial activity of plant extract. All procedures involved in this preparation were done under strict aseptic conditions to avoid contamination during the study. All glassware, including beaker, volumetric flask, dropper, measuring cylinder, pipette, conical flask, laboratory glass bottles, were autoclaved at 121 °C for 20 min prior to use. *Argemone mexicana* Linn. Ethanol and aqueous extracts were tested against *Staphylococcus aureus*, *E. coli*, *Bacillus substilis*, and candida albicans, the common and main skin infection-causing bacteria by agar well diffusion method. The extracts solutions were prepared by using 10% DMSO as a solvent [19, 25]. The culture of *Staphylococcus aureus* was prepared in nutrient agar medium slants and incubated at 37 °C for 24 h under aerobic conditions. The culture of Candida albicans was prepared in Potato dextrose Agar medium slants and incubated at 37 °C for 48 h under aerobic conditions. The culture of Escherichia coli was prepared in nutrient agar medium slants and incubated at 35 °C for 24 h under aerobic conditions. The culture of *Bacillus substilis* was prepared in nutrient agar medium slants and incubated at 37 °C for 24 h under aerobic conditions. Pure culture of bacteria from the plate was suspended in sterile saline to get 108 cells per ml by comparing McFarland tube number 0.5. This suspension was swabbed on the surface of solidified agar plates. Agar plate was punched with a sterile cork borer of 8 mm size, and 100 µL of *Argemone mexicana* Linn ethanol and aqueous extracts of various concentrations (100 mg/mL, 200 mg/mL, and 300 mg/mL) were poured with micropipette in each well. The plates were allowed to stand for 30 min. Then plates of this aerobic bacterium S.aureus were incubated at 37 °C for 24 h under aerobic conditions. Plates of aerobic bacterium *E. coli* were incubated at 35 °C for 24 h under aerobic conditions. Plates of aerobic bacterium *B, subtilis* were incubated at 37 °C for 24 h under aerobic conditions. Plates of Candida albicans were incubated at 25 °C for 48 h under aerobic conditions. The positive control Tetracycline discs (30 µg/mL) were also used

simultaneously. The antibacterial activity of plants was estimated by measuring the diameter of the zone of inhibition in mm.

All well diffusion tests were performed in three separate experiments [19, 20, 21, 24].

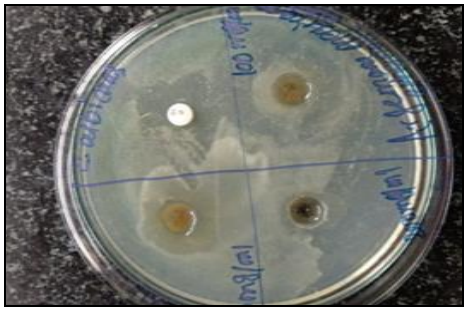


FIG. 1: AM ETHANOL EXTRACT AGAINST *C. ALBICANS*

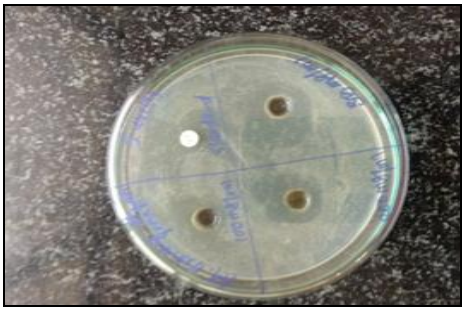


FIG. 2: AM AQUEOUS EXTRACT AGAINST *S. AUREUS*

FIG. 3: AM ETHANOL EXTRACT AGAINST *E. COLI*

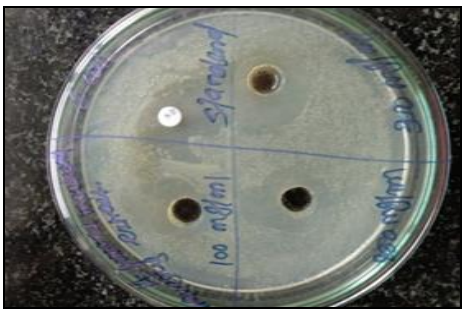


FIG. 4: AM AQUEOUS EXTRACT AGAINST *E. COLI*

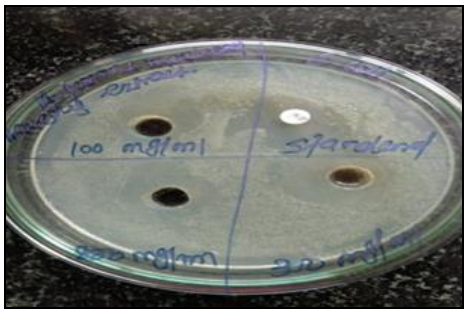


FIG. 5: AMAQUEOUS EXTRACT AGAINST *E. COLI*

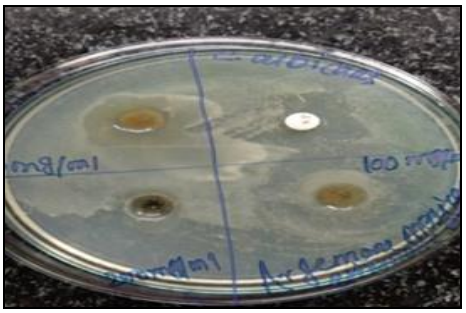


FIG. 6: AM ETHANOL EXTRACT AGAINST *C. ALBICANS*

MIC Value Determination: Weighed amount of Muller Hinton Agar is added to 100 ml distilled water and sterilized at 121 °C for 15 min. Poured in sterile test tubes in a slanting position, agar is allowed to set at ambient temperature and used. Minimum inhibitory concentration determined by Micro dilution assay.

The minimum inhibitory concentration was the lowest concentration of the compound to inhibit the growth of microorganisms. Different concentrations of the extracts (200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 20 mg/ml) were prepared. 0.1 ml of standardized test organism of Controls was equally setup by using solvents and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration. The MIC values of ethanol extract was found to be 20 mg/ml and for aqueous extract 25 mg/ml [22, 23].

Fourier Transform Infrared Spectrophotometer (FTIR): Concentrated herbal extracts of leaves of *Argemone mexicana* Linn were used for FTIR analysis in parkin Elmer.

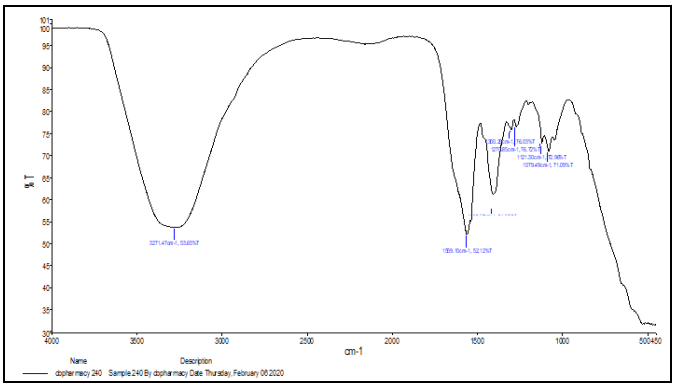
Model no L1600401 spectrum Two DTGS Serial Number 107435, the UK with a scan range from 400 to 4000 cm^{-1} as per instrument manual.

TABLE 2: IR GAPH OF *ARGEMONE MEXICANA* LINN AQUEOUS EXTRACTS

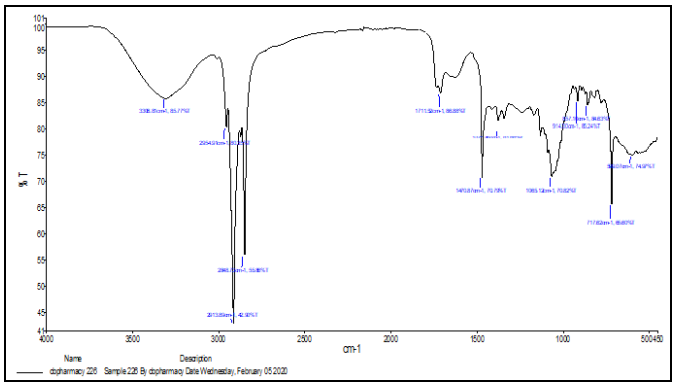
Peak Number	X (cm^{-1})	Type of functional group	Y (%T)
1	3271.47	(O-H stretching of alcohol)	53.65
2	1559.10	(C-H stretching of Aromatic ring)	52.12
3	1406.79	(C-H bending of methyl group)	61.25
4	1300.20	(C-O stretching of primary alcohol)	76.03
5	1270.85	(C-O stretching of Carboxylic acid)	76.72
6	1121.30	(C-O stretching of alcohols)	72.96
7	1079.49	(C-O stretching of alcohols)	71.09

TABLE 3: IR GRAPH OF *ARGEMONE MEXICANA* LINNETHANOL EXTRACTS

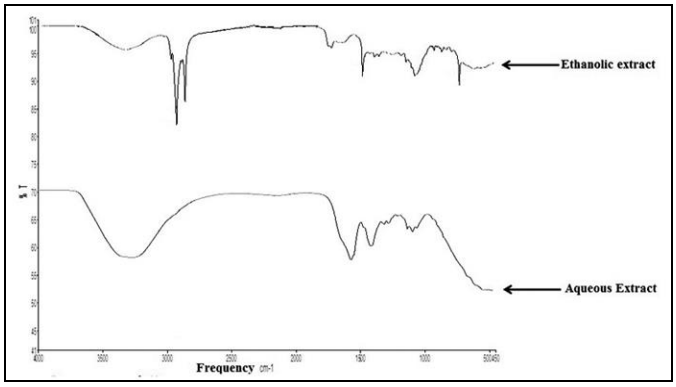
Peak Number	X (cm-1)	Type of functional group.	Y (%T)
1	3306.81	(O-H stretching of alcohol)	85.77
2	2954.91	(C-H stretching of Carboxylic acid)	80.35
3	2913.89	(O-H stretching of Carboxylic acid)	42.90
4	2848.73	(C-H stretching of alkane)	55.86
5	1711.52	(C=O stretching of Carboxylic acid)	86.88
6	1470.87	(C-H bending of methyl group)	70.79
7	1377.46	(O-H bending of alcohol)	81.66
8	1065.12	(C-O stretching of alkyl aryl ether)	70.82
9	914.30	(C-C stretching of aromatic compounds)	85.24
10	857.10	(Unknown)	84.63
11	717.62	(Unknown)	65.60
12	599.07	(Unknown)	74.97



GRAPH 1: IR GRAPH OF ARGEMONE MEXICANA LINNAQUEOUS EXTRACTS



GRAPH 2: IR GRAPH OF ARGEMONE MEXICANA LINNAQUEOUS EXTRACTS



GRAPH 3: IR GRAPH OF ARGEMONE MEXICANA LINNAQUEOUS AND ETHANOL EXTRACTS

DISCUSSION: The plant materials were extracted by using the Soxhlet apparatus. The phytochemical screening of obtained extract was performed to know the presence of various secondary metabolites and is tabulated as **Table 1.** The antibacterial susceptibility was measured by using the agar well diffusion method. *Argemone mexicana* Linn was tested against Staphylococcus aureus ATCC NO 2079, *Bacillis substilis* ATCC NO2192, Candida albicans ATCC NO 3557, *Escheria col*i ATCC NO 8739.

TABLE 4: RESULTS OF ANTIMICROBIAL ACTIVITY OF ARGEMONE MEXICANA LINN EXTRACT

Microorganisms	Zone of inhibition in mm						
	***Argemone Mexicana* Linn (Aqueous extract)**			***Argemone Mexicana* Linn (Ethanol extract)**			**Standard**
Concentrations	100mg/ml	200mg/ml	300mg/ml	100mg/ml	200mg/ml	300mg/ml	30µg/mL
S.aureus	5.1±0.02	5.3±0.2	6.9±0.13	16.7±0.11	17.6±0.08	18±0.09	19±0.09
B.substilis	8.6±0.21	9.2±0.31	9.9±0.23	14.3±0.21	14.9±0.04	15.3±0.03	16.3±0.04
E.coli	6.3±0.12	6.8±0.22	7.8±0.16	15.8±0.19	16.7±0.09	17.2±0.01	17.2±0.02
C.albicans	8.3±0.2	8.9±0.23	9.4±0.21	11.6±0.7	12.8±0.3	13.9±0.1	14.9±0.1

Argemone mexicana Linn ethanol extract showed the zone of inhibition of 16.7±0.11, 17.6±0.08, 18 ±0.09 at concentrations of 100 mg/mL, 200 mg/mL and 300 mg/mL The *Argemone mexicana* Linn aqueous extract showed the zone of inhibition of 5.1± 0.02, 5.3 ± 0.2, 6.9 ± 0.13 at a concentration of 100 mg/mL, 200 mg/mL, 300 mg/mL against *S. aureus. Argemone mexicana* Linn ethanol extract showed the zone of inhibition of 14.3±0.21, 14.9±0.04, 15.3 ±0.03 at concentrations of 100 mg/mL, 200 mg/mL, and 300 mg/mL The *Argemone mexicana* Linn aqueous extract showed the zone of inhibition of 8.6±0.21, 9.2 ± 0.31, and 9.9±0.23 at a concentration of 100 mg/mL, 200 mg/mL, and 300 mg/mL against *Bacillus Substilis. Argemone mexicana* Linn ethanol extract showed the zone of inhibition of 15.8±0.19, 16.7±0.09, 17.2±0.01 at a concentration of 100 mg/mL, 200 mg/mL, and 300 mg/mL The *Argemone mexicana* Linn aqueous extract showed the zone of inhibition of 6.3± 0.12, 6.8 ± 0.22, 7.8 ± 0.16 at a concentration of 100 mg/mL, 200 mg/mL and 300 mg/mL against *E. coli. Argemone mexicana* Linn ethanol extract showed the zone of inhibition of 11.6±0.07, 12.8 ±0.23 and 13.9±0.31 at concentrations of 100 mg/mL, 200 mg/mL and 300 mg/mL The *Argemone mexicana* Linn aqueous extract showed the zone of inhibition of 8.3±0.2, 8.9±0.23, 9.4±0.21 at a concentration of 100 mg/mL, 200 mg/mL and 300 mg/mL against *Candida albicans*. Tetracycline disc (30µg/mL) was used as positive control and the zone of inhibition shown by standard is represented in **Fig. 4.**

FTIR Studies: Ethanol Extracts of *Argemone Mexicana* Linn: The vibrational assignments, intensities and wave number (cm-1) of dominant peak were obtained from absorption spectra. The more intense band occurring at 3306.81cm-1,2954.91 cm-1, 2913.89 cm-1, 2848.73 cm-1, 1711.52 cm-1, 1470.87 cm-1 and 1377.46 cm-1 corresponding to O-H/ C-O str / C-H/C=O stretching, bending, vibrations respectively indicate the presence of alcohol, Phenol, amides, amino acids, meta substituted compounds. The characterstics band at 1065.12 cm^{-1}indicating (C-O str) stretching of alky aryl ether, primary alcohol in ethanol extracts of *Argemone mexicana* Linn**.**

Aqueous extracts of *Argemone Mexicana* Linn: Interestingly observation of aqueous extracts of this plant shows bands occurring at 3271.47 cm-1, indicating the presence of alcohol and phenol (O-H) groups, at 1559.10 cm-1 for (C=C) indicating stretching of an aromatic group, at 1406.79 cm-1 indicating the presence (C-H) bending of methyl group, at 1300.20 cm^{-1},1121.30 cm^{-1},1079.49 cm^{1} indicating (C-O str)stretching of primary alcohols, at 1270.85 cm^{-1}indicating (C-O str)stretching of carboxylic acid [17].

CONCLUSION: The present study supports to traditional uses of this plant in the treatment of some diseases as broad spectrum antimicrobial agent. However, the crude extracts of the plant need to be further purified through isolation and identification of the responsible compounds for antimicrobial activity. The presence of characteristic functional groups Carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens are responsible for various medicinal properties of Argemone mexicana Linn so it contains high therapeutic content. The results from this study indicate that the both extract of this plant having high therapeutic value. Ethanol extract of Argemone mexicana Linn possesses significant antimicrobial activity as compared to aqueous extract. In future, it is used for the treatment of various diseases. It is seen that the main chemical constituent of Argemone mexicana Linn is quercetin from the flavonoids group of polyphenol should possesses well known antimicrobial activity which serve as a main pharmaceutical product in cellulitis, impetigo, eczema, inflammation, and

other skin diseases. This study renders easily available weed Argemone mexicana Linn leaves as medicinal plant, which can be used as alternative medicines to conventional antibiotics which will be a great help in developing countries to fight against skin diseases.

Plant Authentication: The plant parts were identified by Dr. R. M. Mullani, department of botany, SRTMU Nanded. Voucher specimens (Voucher ID SRTMUH.S.L.S.BOTH-105) were deposited in the herbarium section of school of life sciences, SRTMU Nanded.

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