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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF PROSOPIS CINERARIA L.

Nathiya P.*[1], Ramesh S.[2] and N. Nithiya[3]

[1,2,3]Department of Botany, Nehru Memorial College (Autonomous) Puthanampatti -621 007, Trichy. Dt, Tamil Nadu (Affiliated to Bharathidasan University, Tiruchirappalli - 620 024).

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***Corresponding Author**
Nathiya P.
Department of Botany,
Nehru Memorial College
(Autonomous)
Puthanampatti -621 007,
Trichy. Dt, Tamil Nadu
(Affiliated to Bharathidasan
University, Tiruchirappalli -
620 024).

ABSTRACT

Prosopis cineraria is locally known as Khejri one of the most common tree in Indian desert and belonging to family Fabaceae. The aim of this study was to carry out for identification of bioactive compounds from the leaves of ethanolic extract of Coleus aromatics by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis revealed the presence of various compounds like 1,2-benzenedicarboxylic acid, diethyl ester, Phytol, Octadecenal, Dibutyl phthalate, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, 9,12,15-Octadecatrienoic acid, ethyl ester and solanesol in the ethanolic extract of Coleus aromaticus. These findings support the traditional use of Coleus aromatics in various disorders. The present study was carried out to investigate the antibacterial activity present in the leaves, of *Prosopis cineraria* in Agar well diffusion method. Six bacterial strains were used as test microbes. The study was revealed the plant *Prosopis cineraria* showed the inhibitory zone against the bacteria. The highest zone of inhibition was showed by *P. cineraria* leaves against *Aeromona shydrophilla* (13.3 ±0.92 mm) and by *P. cineraria* leaves against *Staphylococcus aureus* (9 ±1.10mm). *E.coli (*9.33 ± 2.05), *Streptococcus faecalis*, (12.9 ±0.41) *Pseudomonas aeruginosa* (3.6 ±0.92 mm).

KEYWORDS: *Prosopis cineraria*, Fabaceae, Medicinal uses, GC-MS, Antibacterial activity.

1. INTRODUCTION

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. (Vandna Pathak, 2016). *Prosopis cineraria* L. Druce belongs to family Fabaceae commonly known as "Khejari" It is also known as the wonder tree and the king of desert. It is commonly found in dry and arid regions of north western and southern India. (Ashish Kumar Pareek,*et al,* 2015). Phytochemical investigations of the leaves on plant resulted in the presence of hydrocarbons, phenol, alkaloids, proteins, carbohydrates, flavonoids, saponins and tannins derivatives (Chaudhary, 2018, Grag, 2013)

Pharmaceutical application *P.cineraria* such as pain, high cholesterol level, diabetes, anemia, kidney, disorders (Preeti Khandelwal, 2016). The leaf is high nutrient content like carbohydrates, proteins, fats, minerals and vitamins. Leaf paste is applied on boils and blisters, including mouth ulcers in livestock. The smoke of the leaves is considered good for eye troubles. Leaf extracts of *P. cineraria* have shows antibacterial, anti-hyperglycemic, anti-hyperlipidemic and antioxidative activities. It is also used by native healers to manage multiple ailments including gastrointestinal, respiratory, and cardiovascular disorders. (Sachdeva, 2014, Shruti Malik, *et al*, 2012).The stem bark has folkloric repute to possess anti-inflammatory, anti rheumatic, tonic, and vermifuge properties present in the plant. It is used in the treatment of anxiety, asthma, bronchitis, dyspepsia, fever, dysentery, leprosy, piles, watering of the mind and tumors. (Sharma, 2012 Sharma, 2013).

2. MATERIALSANDMETHODS

Sample collection

Prosopis cineraria (L.) popularly known as Vanni tree one of the important medicinal plant. In the present study the plant leaves was collected in polythene bags during the November 2024 from in around **Pachamalai hills and Trichy** District Tamil nadu India.

Preparation of plant extract

The leaves were washed several times with distilled water to remove the traces of impurities. They were dried at room temperature and coarsely powdered. The powder was different extracted with for 48 hours. A semi solid extract was obtained after a complete elimination of solvent under reduced pressure. The leaves extract was stored in refrigerator until used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column 30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane, operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed split ratio of 10:1 injector temperature 250 ºC ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC isothermal for 2 min, with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. Mass spectra were taken at 70eV a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0.

ANTIBACTERIAL ACTIVITY

AGAR WELL DIFFUSION METHOD

The antibacterial present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. Nutrient Agar medium, Nutrient broth, Whatman filter paper No. 1, Gentamicin antibiotic solution, test samples, test tubes, beakers conical flaks, spirit lamp, double distilled water and petri-plates.

Culture medium (Nutrient Agar Medium)

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (Hi Media) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30ml/plate) while still molten.

Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Petri plates containing 20ml nutrient agar medium were seeded with 24hr culture

of bacterial strains *(Aeromonas hydrophila, Staphylococcus aureus, E.coli, Streptococcus faecalis, Streptococcus faecalis, Proteus vulgari and, Pseudomonas aeruginosa,*. Well were cut and different concentration of sample A (500 µg/ml, 250 µg/ml, 100 µg/ml and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

3. RESULTS AND DISCUSSION

Plant have an almost ability to synthesize many number of substances is present. GC-MS analysis fifty six compounds were identified in Coleus aromatic us by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented. The GC-MS analysis revealed the presence of various compounds like 1,2-benzenedi-carboxylic acid, di-ethyl ester, phytol, oct-adecenal, di-butyl phthalate, 2- hexadecen-1-ol, 3,7,11,15-tetramethyl, hexa-decanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, (z,z,z), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z) and solanesol represents the activity of phytocomponents identified in the ethanolic extracts of the Coleus aromaticus leaves by GC-MS. in (Table-1 and Fig-1).In the previous study he screened for phytochemical compounds present in *Prosopis cineraria* and showed the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins and steroids. (Kolapo, 2009, Grag, 2013).The chemical constituent of plants is desirable because such information will be value for synthesis of complex chemical substances. It is recommended as a plant of phyto pharmaceutical importance on account of the abundance level of major phyto compounds that may be utilized by drug designer's following appropriate isolation and characterization procedures for the active compound present in the plant. (Pavithra,*et al* 2020, Malic,2007.).

Preliminary screening of antimicrobial potential was evaluated by using Agar well diffusion method on different extracts of dried unripe pods of *Prosopis cineraria* presented in (Table-2 and Fig-2) respectively. Eethanolic extract shows significant results on all pathogens whereas no activity was recorded by ethyl acetate extract. Among the tested six gram negative bacteria, *Staphylococcus aureus* (9.9 ±0.43) was more susceptible to methanol, chloroform and aqueous extracts. Maximum zone of inhibition (13.0±1.0) was observed against *Aeromonas hydrophilla* in ethanolic extract, whereas (16.0±1.0) in chloroform extract and

(5.3±0.57) in aqueous extract. The minimum zone of inhibition (9.6±0.57) was showed by methanol extract in *E.coli.* (9.33 ±1.0) Standard antibiotics, Gintamycin was taken as a positive control. It was observed that ethanol the extract showed better results against all pathogens in comparison to standard antibiotics. (Table-2 and Fig -2).Earlier the antimicrobial properties were reported by researchers from stem, bark and leaflets of *Prosopis cineraria* and even from root, stem, bark, pods of the different species of *Prosopis julifera* and *Prosopis africana*. (Velmurugan, 2011). Therefore, this was the first attempt when dried unripe pods of *Prosopis cineraria* were screened for antimicrobial potential. (Tarachand, *et al* 2012, Velmurugan, 2010).

Table 1: Phytochemical studies on GC-MS Analysis of leaves extracts of *Prosopis cineraria* L.

Start Tm	End Tm	Ret.Time	StartRT	EndRT	Search	Name
8.26	8.27	8.265	8.235	8.3	Done	1-DODECENE
8.89	8.9	8.895	8.86	8.935	Done	Benzothiazole
9.085	9.095	9.09	9.05	9.175	Done	Benzaldehyde, 4-(1-methylethyl)-
9.455	9.465	9.46	9.43	9.495	Done	HEXADECANE, 2,6,10,14-TETRAMETHYL-
9.74	9.75	9.745	9.7	9.815	Done	Phenol, p-tert-butyl-
10.495	10.505	10.5	10.465	10.54	Done	3-CYCLOHEXENE-1-METHANOL, . ALPHA.,.ALPHA.,4-TRIMETHYL-, ACETATE
11.055	11.065	11.06	11.02	11.1	Done	1-Pentadecene
11.16	11.17	11.165	11.135	11.2	Done	TETRADECANE
11.795	11.805	11.8	11.765	11.84	Done	QUINOLINE, 1,2-DIHYDRO-2,2,4-TRIMETHYL-
12.3	12.31	12.305	12.28	12.335	Done	Eicosane
12.44	12.45	12.445	12.42	12.475	Done	Pentadecane
12.55	12.56	12.555	12.475	12.585	Done	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-
13.54	13.55	13.545	13.5	13.565	Done	Diethyl Phthalate
13.57	13.58	13.575	13.565	13.61	Done	1-Heptadecene
13.66	13.67	13.665	13.61	13.695	Done	OCTADECANE
14.815	14.825	14.82	14.785	14.85	Done	Heptadecane
15.13	15.14	15.135	15.075	15.18	Done	BENZENE, ETHYLPHENOXY-
15.535	15.545	15.54	15.46	15.58	Done	TETRADECANOIC ACID
15.765	15.775	15.77	15.735	15.805	Done	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis-
15.835	15.845	15.84	15.805	15.88	Done	1-OCTADECENE
15.91	15.92	15.915	15.885	15.935	Done	Octadecane
16.04	16.05	16.045	15.935	16.095	Done	BENZENE, ETHYLPHENOXY-
16.945	16.955	16.95	16.93	17.03	Done	Heneicosane
17.06	17.07	17.065	17.03	17.1	Done	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
17.19	17.2	17.195	17.1	17.245	Done	Hexadecanoic acid, methyl ester
17.485	17.495	17.49	17.46	17.565	Done	Benzothiazole, 2-(2-hydroxyethylthio)-
17.645	17.655	17.65	17.565	17.73	Done	n-Hexadecanoic acid

17.885	17.895	17.89	17.835	17.925	Done	n-Tetracosanol-1
18.76	18.77	18.765	18.735	18.795	Done	n-Tetracosanol-1
18.895	18.905	18.9	18.875	18.93	Done	Octacosane
19.125	19.135	19.13	19.08	19.16	Done	Methyl stearate
19.185	19.195	19.19	19.16	19.22	Done	[1,1'-BICYCLOHEXYL]-1-CARBOXYLIC ACID, 2-(DIETHYLAMINO)ETHYL ESTER
19.445	19.455	19.45	19.42	19.47	Done	Linoleic acid ethyl ester
19.5	19.51	19.505	19.47	19.55	Done	9-OCTADECENOIC ACID (Z)-, ETHYL ESTER
19.695	19.705	19.7	19.63	19.73	Done	PHENOL, 4,4'-(1-METHYLETHYLIDENE)BIS-
19.755	19.765	19.76	19.73	19.785	Done	Behenic alcohol
19.805	19.815	19.81	19.785	19.84	Done	DOCOSANE
19.87	19.88	19.875	19.84	19.915	Done	Eicosyl acetate
20.295	20.305	20.3	20.27	20.34	Done	N ,N-DIMETYLPALMITAMIDE
20.615	20.625	20.62	20.59	20.65	Done	(3R,3AS,4S)-3-HYDROXY-3-ISOPROPYL-6,8A-DIMETHYL-1,2,3,3A,4,5,8,8A-OCTAHYDRO-4-AZULENYL 4-METHOXYBENZOATE
20.68	20.69	20.685	20.65	20.725	Done	Pentacosane
21.205	21.215	21.21	21.17	21.25	Done	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-
21.43	21.44	21.435	21.4	21.465	Done	Hexanedioic acid, bis(2-ethylhexyl) ester
21.52	21.53	21.525	21.465	21.555	Done	Tetracosane
21.58	21.59	21.585	21.555	21.62	Done	Eicosyl acetate
21.685	21.695	21.69	21.625	21.735	Done	Phenol, 2,4-bis(1-phenylethyl)-
21.82	21.83	21.825	21.76	21.855	Done	Phenol, 2,4-bis(1-phenylethyl)-
21.875	21.885	21.88	21.855	21.92	Done	1,3-Diphenyl-1-(2-hydroxyphenyl)butane
22.02	22.03	22.025	21.92	22.085	Done	4-(1,3-Diphenylbutyl)phenol
22.29	22.3	22.295	22.21	22.315	Done	Phenol, 2,4-bis(1-phenylethyl)-
22.325	22.335	22.33	22.315	22.37	Done	Pentacosane
22.425	22.435	22.43	22.37	22.47	Done	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
22.585	22.595	22.59	22.56	22.62	Done	BIS(2-ETHYLHEXYL) PHTHALATE
23.04	23.05	23.045	22.995	23.07	Done	2-(2H-BENZOTRIAZOL-2-YL)-4-(1,1,3,3-TETRAMETHYL BUTYL)PHENOL
23.1	23.11	23.105	23.07	23.14	Done	HEXACOSANE
23.655	23.665	23.66	23.615	23.695	Done	4,4'-((p-Phenylene)diisopropylidene)diphenol
23.92	23.93	23.925	23.875	23.975	Done	HEXACOSANE
24.495	24.505	24.5	24.47	24.53	Done	2-Methylhexacosane
24.875	24.885	24.88	24.825	24.93	Done	HEXACOSANE

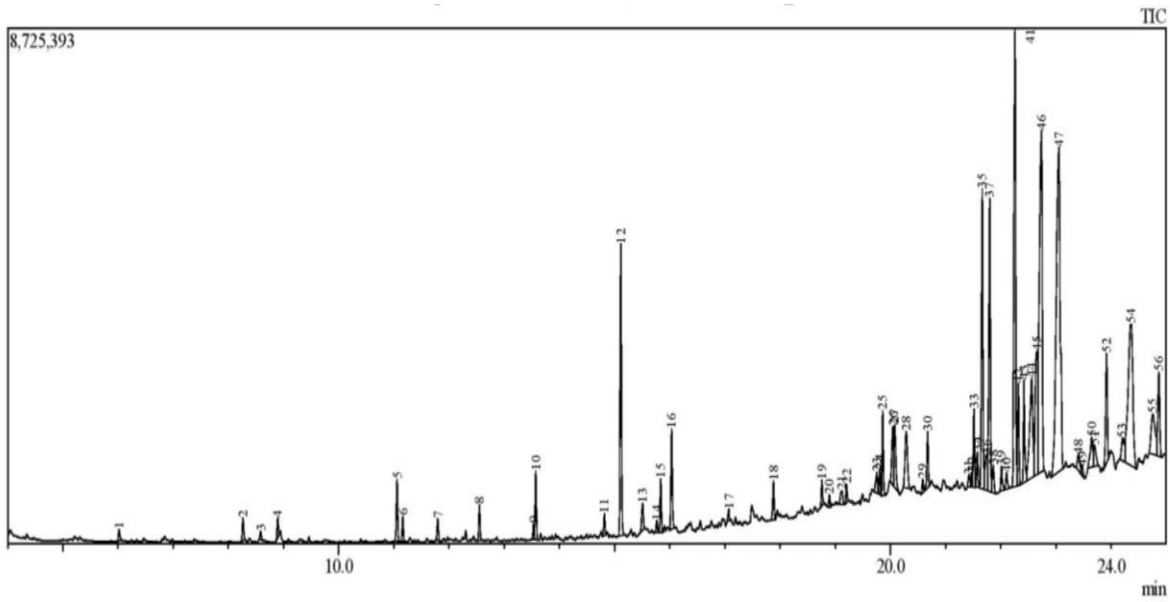
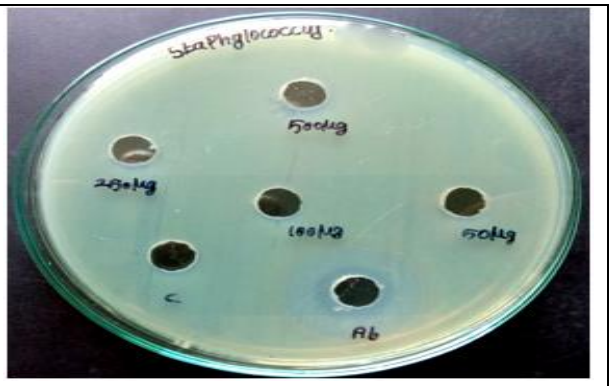
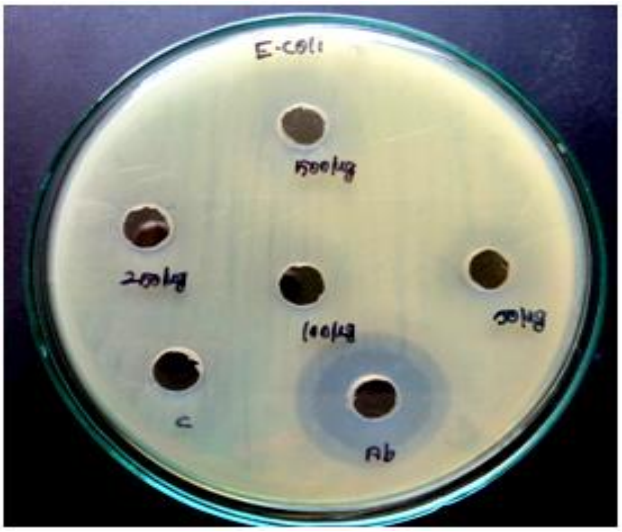
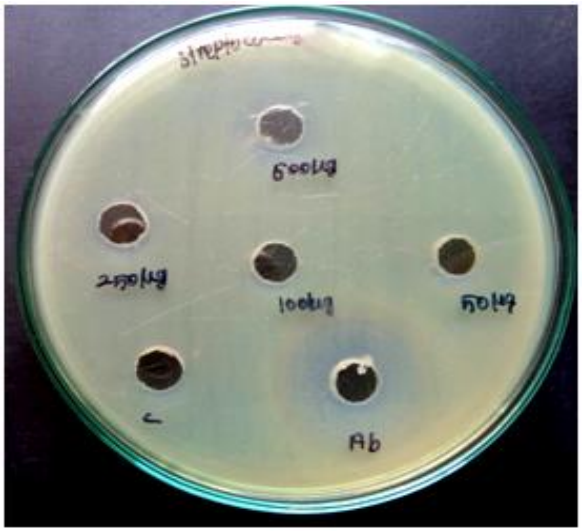
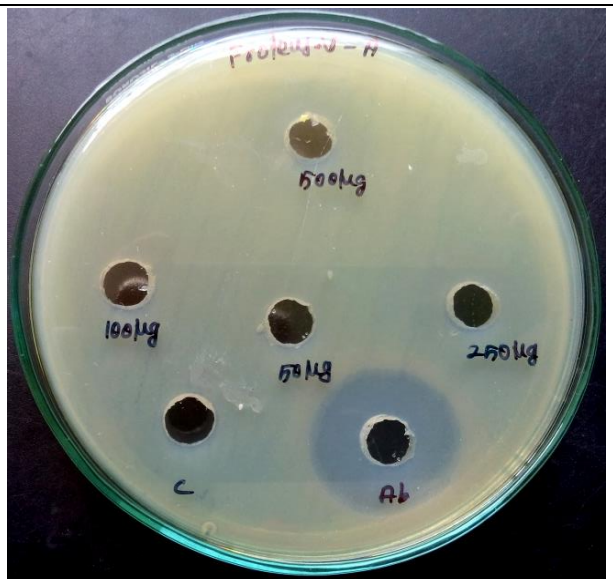
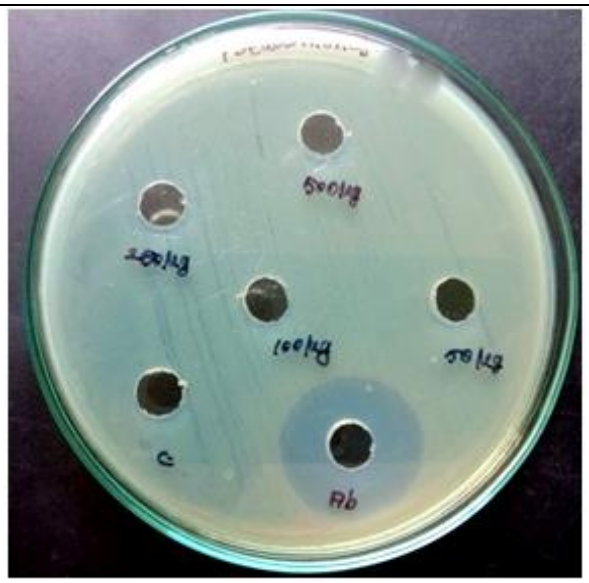


Fig -1 Phytochemical studies on GC-MS Analysis of leaves extracts of *Prosopis cineraria* L.

	
Aeromona shydrophilla	***Staphylococcus aureus***
	
E.coli	***Streptococcus faecalis***



Proteus vulgaris



Pseudomonas aeruginosa

Fig 2: Means zone of inhibition obtained from sample against bacteria *Aeromonas hydrophila, Staphylococcus aureus, E.coli, Streptococcus faecalis, Proteus vulgaris*, and *Pseudomonas aeruginosa.*

Table 2: Means zone of inhibition obtained from sample against bacteria *Aeromonas hydrophila, Staphylococcus aureus, E.coli, Streptococcus faecalis, Proteus vulgaris*, and *Pseudomonas aeruginosa.*

S.NO	Name of the test micro organisms	Zone of the inhibition (mm)			
		500µg/ml	250 µg /ml	100 µg /ml	50 µg /ml
1	*Aeromonas hydrophilla*	13± 1.63	9.7 ± 0.57	6.33± 2.05	5.66 ± 2.49
2	*Staphylococcus aureus*	9.9 ±0.43	4 ± 1.63	3.93 ± 0.09	2.93 ± 0.09
3	*E.coli*	9.33 ± 2.05	7.33 ± 1.24	7.16 ± 0.24	2.93 ± 0.09
4	*Streptococcus faecalis*	12.9 ±0.41	3.66 ±1.69	2.8 ± 0.21	3.39 ± 0.28
5	*Proteus vulgaris*	4.33 ± 1.24	7.83 ±0.41	5.83± 0.24	3.39 ± 0.28
6	*Pseudomonas aeruginosa*	3.66 ± 1.69	5.93 ± 0.09	7.8 ± 0.29	4.56 ± 0.32

CONCLUSION

Prosopis cineraria commonly used for various kinds of diseases is regarded as a plant of high medicinal value because of its leaves and bark. This plant was suggested various therapeutic use of plant were reported such as antidepressant, anticancer and anti-inflammation. *Prosopis cineraria* deals with a of phytochemical constituents including alkaloids, carbohydrates, phytosterols, saponins, phenols, tannins, flavonoids, terpenoids, phlobatannins, protein and free amino acids are present in the leaf material. The plant can be recommended extended for future investigation into the field of phytochemistry, ethnobotany, pharmacology and other biological action for drugs are present.

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