Pharmacogn. Commn. 2019; 9(2): 54-58
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Characterization of the, Antimicrobial, Antioxidant Activity of Proteins from *Prosopis cineraria* Leaves

Mayank Kulshreshtha[1,*], Karuna Shanker Shukla[1], Garima Tiwari[2], Manjul Pratap Singh[1]
[1]School of Pharmacy, Babu Banarasi Das University, Lucknow, Uttar Pradesh, INDIA
[2]Goel Institute of Pharmacy and Sciences, Lucknow, Uttar Pradesh, INDIA.

ABSTRACT

Introdutction: The present study was designed to screen out the different proteins from *Prosopis cineraria* (Leaves), known as Shammi (Family Fabaceae) and characterize them by IR, NMR and mass spectroscopy and evaluation of their antimicrobial potential against various microbial species. **Mehtods:** Different extracts of leaf were treated with different reagents for the presence and absence of metabolites. Antimicrobial effect of the proteins were explored against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus tubingensis*. The disc diffusion method was used for determination of antimicrobial activity. Analytical analysis included IR, NMR and Mass spectroscopy. **Reults:** Phytochemical analysis of different extracts showed the presence of different primary and secondary metabolites The range of zone of inhibition were found to be from 1.4±0.01mm to 14.2±0.18 mm. The maximum antimicrobial activity was found for 50 KDa protein against *Pseudomonas aeruginosa.*

Key words: *Prosopis cineraria*, Phytochemical analysis, Antimicrobial, Antioxidant, Monograph, Protein.

Correspondence:
Prof. Mayank Kulshreshtha
Assistant Professor, Department of Pharmacology, School of Pharmacy, Babu Banarasi Das University, Lucknow, Uttar Pradesh-226028, INDIA.
E-mail: professormayank@gmail.com
Phone no: +91-9897083663
DOI: 10.5530/pc.2019.2.12.

INTRODUCTION

Medicines from herbal plants are gaining importance in health care systems and play a very important role in pharmacological treatments.[1] Medicines and healthy food from herbs often have less side effects than synthetic medicines.[2] Various analytical methods are used to establish for their qualitative and quantitative estimation.[3]

Prosopis cineraria (*P. cineraria*), family- Fabaceae, an indigenous plantmentioned in Ayurveda with several clinical benefits.[4] Locally it is called "Kalpavriksha"[5] and its use is common to the Hindu Bhagavatas, the Jains and the Buddhists.[6] Mythology has refereed to this tree as the Shammi tree of lord Rama and Laxamana because proportedly they placed their swords and weapons on this tree.[7] The genus *Prosopis* comprises 44 species distributed mainly in Southwest Asia, Africa and the America, ranging from western North America south to Patagonia.[8] Vernacular names are Khejri (Hindi and Sanskrit), Janti/Loong tree (Rajasthani), Jand (Punjabi), Sami (Gujarat), Sumri (Tamil) and Jammi (Telugu) and in Sind it is known as Kandi.[9] Leaves of this species contain campesterol, cholestrol, sitosterol, stigmasterol, actacosanol, hentriacontane andmethyl docosanoate.[10,11] These compounds possess potent antioxidant, hypoglycemic and thyroid inhibiting properties.[12] Fresh leaf juice mixed with lemon juice is used for dyspepsia.[13] The extract of crushed pods is used for earache, toothache, pain relief from fractured bones while aqueous extract of bark and leaves are useful in wounds if applied externally.[13,14] *P. cinerarias* is reported to have various pharmacological activities including analgesic and antipyretic, antihyperglycemic, antioxidant, anti-hypercholesterolemic, antitumor, no tropic, anticonvulsant effects etc. Due to the presence of various primary and secondary metabolites.[15-28]

There is a lack of scientific data on the proteins of this species and there analytical, antimicrobial and antioxidant activities. Hence, the present study was undertaken to assess the phytochemical content and screening the proteins for their microbiological and antioxidant properties as per WHO and standard guidelines. This scientific research data may serve as a reference for future researches, preparation of various herbal formulations, analytical analysis, protein based studies and preparing monographs about this plant.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The leaves of *P. cineraria* were collected from National Botanical Research Institute, Lucknow, India in July 2015 and authenticated by Dr. Sunita Garg, NISCAIR, Delhi (Ref No. NISCAIR/RHMD/2015/2862/55-2).

Reagents

SDS-PAGE Teaching kit was purchased from Medox-Bio™, Chennai (Catlog no. MX-1180-02). DPPH was purchased from SDFCL, Mumbai. Ethanol purchased from Changshu Yangyuan chemical, China. Azithromycin purchased from local market of Lucknow. Ciprofloxacin and Fluconazole are provided as a free gift sample from Alkem Pharmaceuticals and Sun Pharmaceuticals respectively. All the chemicals were analytical grades.

Extraction of Leaves

Extractions of the leaves was carried out according to standard procedures.[29] The plant material was dried in a light free area and powdered using a mechanical grinder. The powdered (25.0 g) plant materials was dried and treated with different solvents (Water, ethanol and methanol). Extracts were then conserved in sterile bottles in refrigerated conditions for further use. The extracts were kept at 2 to 4°C. The crude extracts were used for further investigation of antimicrobial properties.[29]

Identification of Primary and Secondary Metabolites by Phytochemicals Screening

Identification of carbohydrate, alkaloids, flavonoids, tannins, proteins, terpenoids etc was achieved by standard chemical tests.[30-32]

Screening of different proteins from ethanolic extract of *P. cineraria* through SDSPAGE electrophoresis

Resolving gel, stacking gel were prepared and sample preparation was done by 20-40 micro grams total protein per mini-gel loaded in wells

then added equal volume of both sample and lamelli buffer. Afterwards, incubated each sample for 5 min at 95-100°C. Now 10 ml mixture loaded in the well (Mini gel 15 well comb and 1mm spacers) and combs removed combs from the gel. After that, it was casted and placed it in the electrophoresis chamber filled with electrophoresis buffer. All protein samples were loaded in the gel and applied 70 volts for 3 hrs. After 3 hrs, power was turned off and immediately the proteins were treated with 40% distilled water, 10% acetic acid and 50% methanol to made the proteins insoluble. Again mixture was treated with water and Coomassie Brilliant Blue R-259 and incubated for 4 hrs to overnight at room temperature on a shaker. Transferred the gel to a mixture (67.5% distilled water, 7.5% acetic acid and 25% methanol), placed on shaker and replaced with fresh rinse mixture until the excess dye has been removed and deep blue color was seen. Now the proteins were transferred to the membrane from the gel. Membrane was transferred in to the TBST and again activated in methanol for 30 min, added Ponceau Red and incubated on an agitator for 30 mins. Membrane again washed with in water until the water was clear and the protein bands were well defined. Proteins were separated out by the sharp blade and preserved in a phosphate buffer for future evaluation.[33]

In vitro antimicrobial studies of screened proteins

Antimicrobial studies of the screened proteins have done by agar well diffusion method.[34] Microbial stains procured from Institute of Microbial Technology, Chandigarh. Culture media for the activation of microbes were prepared according to official protocol as given in microbial type culture collection protocol. Proteins were screened against Gram positive bacteria (*Staphylococcus aureus*, MTCC 1430), Gram negative bacteria (*Pseudomonas aeruginosa*, MTCC 2453) and a fungal stain (*Aspergillus tubingensis*, MTCC 2546). Screened proteins dissolved in dimethylsulphoxide (DMSO), concentration range should not be more than 10 % of DMSO (RFCL Limited, New- Delhi).[34]

Antioxidant activity using DPPH (1, 1-diphenyl-2- picryl - hydrazyl) method

DPPH (0.1 mM solution) was dissolved in methanol. From this 1.0 ml solution was added to 3.0 ml of extract solution in water at different concentrations (25, 50, 75, 100 µg/ml). This was incubated at room temperature for 45 min. and the absorbance was measured at 517 nm against the corresponding blank solution. Ascorbic acid (Thomas Chemical Laboratory, Mumbai) was taken as reference. The percentage inhibition of DPPH free radical was calculated based on the control reading, which contained DPPH and distilled water. The % scavenging activity was calculated using the following equation.[35]

DPPH scavenged (%) = {(Absorbance of control – Absorbance of test) / Absorbance of control} × 100

Spectroscopical Analysis of Proteins

IR spectra were recorded on a FT-IR (Shimadzu) in KBr from 400-4000 cm^{-1}. ^{1}HN MR spectra were recorded on JEOL AL 300 FT-NMR spectrophotometer (400 MHz) in $CDCl_3$ using tetramethylsilanae as internal standard. ESI mass spectra were recorded using Agilent 6530 Accurate-Mass Q-TOF.

RESULTS

Phytochemical Analysis of different Extracts

The different extracts were tested with several reagents for the presence of alkaloids, glycosides, tannins, flavanoids, facts and oils, carbohydrates, reducing sugar, proteins, saponin, terpenoids respectively using standard techniques. AEPC (Aqueous extract of *P. cineraria*) shows the presence of flavanoids and proteins in high concentration. In EEPC (Ethanolic extract of *P. cineraria*) showed tannins and flavanoids whereas alkaloids, tannins, flavanoids, proteins, terpenoids in high concentrations (Table 1).[30-32]

Screening of proteins and their antimicrobial effects

Western blotting different proteins detected a number of proteins with molecular weights of 10, 25, 30, 35, 48, 50, 55 and 70 KDa (kDa) (Figure 1). Table 2 shows antimicrobial effects of screened proteins with a gram positive, gram negative and fungal strains. The Zone of Inhibitions (ZOIs) found to between 1.5±0.01mm to 8.1±0.07 mm. Maximum ZOI found to be 8.1±0.07 mm with 33 kda weight protein against *S. aureus* whereas minimum ZOI was 1.5±0.01mm against *A. tubingensis*. These proteins did not have good antifungal properties. *P. aeruginosa* and *S. aureus* are responsible for various diseases like pneumonia, bacterimia, endocarditis, meningitis, etc. If any formulations develop with these proteins so they may be cure the human beings from the above mentioned diseases. In the case of the fungal strain, these protein preparations may

Table 1: Phytochemical Analysis of Different Extracts of *P. cineraria*.

Phytoconstituents	AEPC	EEPC	MEPC
Alkaloids	+	++	+++
Glycosides	+	+	++
Tannins	++	+++	+++
Flavonoids	+++	+++	+++
Fats and oil	+	+	+
Carbohydrates	++	++	++
Reducing sugar	+	+	+
Proteins	+++	++	+++
Saponins	-	-	+
Terpenoids	++	++	+++

AEPC- Aqueous extract of *P. cineraria*, EEPE- Ethanolic extract of *P. cineraria* MEPC-Methanolic extract of *P. cineraria*. Positive sign indicate the presence of various phytoconstituents with respective to concentration like + (good), ++ (better), +++ (best) and negative sign indicate the absence of phytoconstituents.

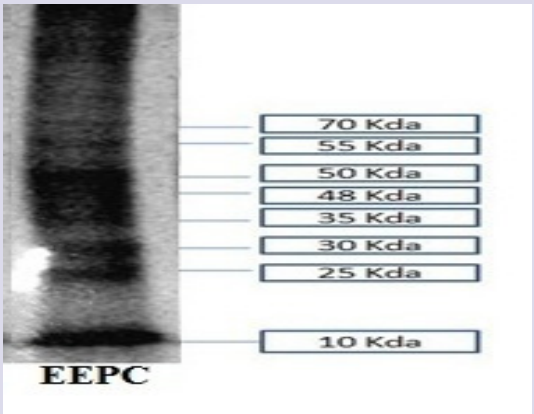


Figure 1: Screening of Protein from EEPC.

give symptomatic relief or as a secondary choice of drug in future. Further researches are needed on these proteins.[36]

Analysis of Proteins by FTIR, ^{1}H NMR and Mass Spectroscopy

All proteins are liquid to semisolids with different colors, melting point between 28°C to 45°C and R_f values were found between 0.55 to 0.79. The IR spectroscopic analysis justified the presence of different functional groups like –N-H, -C=O, amide –C=O, OH, C=O of carboxylic group etc in all screened proteins. These groups are reported to have good antimicrobial property and responsible for curing various diseases like neurological, cardiovascular, gastrointestinal problems etc. Mass spectroscopy proved the molecular weight of compounds in which 10 kda protein has calculated molecular weight is 10 kda but observed molecular ion peaks are ESI-MS: m/z $(M+1)^+$ and M^+. For 25 kda proteins have calculated molecular weight is 25 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+1)^+$. For 30 kda proteins have calculated molecular weight is 30 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+2)^+$. For 35 kda protein has calculated molecular weight is 35 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+1)^+$. For 48 kda protein has calculated molecular weight is 48 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+1)^+$. For 50 kda protein has calculated molecular weight is 50 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+1)^+$. For 55 kda protein has calculated molecular weight is 55 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+2)^+$. For 70 kda protein has calculated molecular weight is 70 kda but observed molecular ion peaks are ESI-MS: m/z M^+ and $(M+1)^+$. ^{1}H NMR characterizations helpful for the establishing of the structure of the compounds and revealed the presence of various protons in which for 10 kda protein, ^{1}H NMR ($CDCl_3$) δ ppm: 9.05 (s, 1H-CO-NH-CO), 9.03 (s,1H, NH). For 25 kda protein, ^{1}H NMR ($CDCl_3$) δ ppm: 9.26 (s, 1H-CO-NH-CO), 9.04 (s,1H, NH). For 30 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 9.32 (s, 1H-CO-NH-CO), 9.01 (s,1H, NH). For 35 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 9.17 (s, 1H-CO-NH-CO), 9.11 (s,1H, NH). For 48 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 8.26 (s, 1H-CO-NH-CO), 8.53 (s,1H, NH). For 50 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 8.27 (s, 1H-CO-NH-CO), 8.11 (s,1H, NH). For 55 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 9.52 (s, 1H-CO-NH-CO), 9.22

Table 2: Antimicrobial activity of Proteins from *P. cineraria* with Different Strains.

Zone of inhibition by different proteins against different strains (in mm)					
Protein Coding	Molecular Weight (kda)	Conc. (µg/ml)	Gram negative strains	Fungal strain	Gram positive strains
			P. aeruginosa	*A. tubingensis*	*S. aureus*
EEPC	10	50	3.2±0.02	2.4±0.02	4.1±0.03
EEPC	25	50	5.5±0.10	2.2±0.01	5.7±0.12
EEPC	30	50	7.3±0.13	NIL	8.1±0.07
EEPC	35	50	6.5±0.11	NIL	6.6±0.12
EEPC	48	50	6.9±0.14	2.1±0.01	7.3±0.13
EEPC	50	50	4.8±0.08	1.5±0.01	5.4±0.06
EEPC	55	50	5.5±0.10	1.6±0.01	5.3±0.05
EEPC	70	50	6.1±0.09	2.2±0.01	6.2±0.06
Ciprofloxacin	Standard	50	15.8±0.24	Nil	14.7±0.21
Azithromycin	Standard	50	10.8±0.14	Nil	10.2±0.06
Fluconazole	Standard	50	Nil	14.3±0.17	Nil

Table 3: Antioxidant activity of Different Proteins of *P. cineraria* using DPPH Method.

Protein Coding and standard	Molecular Weight (kda)	Conc. (µg/ml)	Absorbance	Percentage inhibition	Regression equation	IC_{50}
EEPC	10	25 µg/ml	0.531	40	Y=0.7x + 42 (R^2=0.931)	11.4
		50 µg/ml	0.541	39		
		75 µg/ml	0.543	39		
		100 µg/ml	0.540	38		
EEPC	25	25 µg/ml	0.531	40	Y=0.7x + 41.2 (R^2=0.963)	12.5
		50 µg/ml	0.534	40		
		75 µg/ml	0.537	40		
		100 µg/ml	0.541	39		
EEPC	30	25 µg/ml	0.563	42	Y=0.8x + 43.2 (R^2=0.953)	8.5
		50 µg/ml	0.551	41		
		75 µg/ml	0.542	41		
		100 µg/ml	0.541	40		
EEPC	35	25 µg/ml	0.611	42	Y=0.9x + 45.4 (R^2=0.965)	5.7
		50 µg/ml	0.559	42		
		75 µg/ml	0.554	40		
		100 µg/ml	0.541	39		

EEPC	48	25 µg/ml	0.654	40	Y=0.8x + 46.8 (R^2=0.982)	3.5
		50 µg/ml	0.651	39		
		75 µg/ml	0.594	39		
		100 µg/ml	0.585	37		
EEPC	50	25 µg/ml	0.685	42	Y=0.8x + 44.5 (R^2=0.863)	6.8
		50 µg/ml	0.681	41		
		75 µg/ml	0.679	41		
		100 µg/ml	0.674	40		
EEPC	55	25 µg/ml	0.579	42	Y=0.9x + 48.2 (R^2=0.986)	2.0
		50 µg/ml	0.578	42		
		75 µg/ml	0.574	41		
		100 µg/ml	0.571	40		
EEPC	70	25 µg/ml	0.558	42	Y=0.8x + 47.3 (R^2=0.883)	3.3
		50 µg/ml	0.551	41		
		75 µg/ml	0.498	41		
		100 µg/ml	0.496	39		
Ascorbic acid	standard	25 µg/ml	0.458	49.1	Y=0.33x + 49.35 (R^2=0.959)	1.96
		50 µg/ml	0.462	48.6		
		75 µg/ml	0.465	48.3		
		100 µg/ml	0.467	48.1		

(s,1H, NH). For 70 kda protein ^{1}H NMR ($CDCl_3$) δ ppm: 9.29 (s, 1H-CO-NH-CO), 8.24 (s,1H, NH).

Antioxidant activity of screened proteins

Antioxidant activity of the proteins were determined by DPPH method in which DPPH is a nitrogen based free radical is converted stable molecule dipheny 1- picryl hydrazine. The reduction of DPPH by the extracts due to transferred of hydrogen atom or electrons whereas phenolic compounds have better antioxidant property. Table 3 shows the DPPH radical scavenging activity of different extracts is compared to ascorbic acid. Minimum IC_{50} value found to be 3.5 (Y=0.8x + 46.8, R^2=0.982) with 48 kda protein and maximum IC_{50} value was (Y=0.7x + 41.2, R^2=0.963) 12.5 with 25 kda protein whereas of ascorbic acid has 1.96 (Y=0.33x + 49.35, R^2=0.959).[37,38]

CONCLUSION

In the modern area of world, people are very smart and intelligent. They are depending on the high technologies for daily life but in health care system; they truly depend on herbals due to very low side effects. Present investigation proved screening of various proteins from plant source and it is already reported that protein are main building blocks of the body. Presence of various groups in the proteins justifies the structure of protein and these groups also responsible for curing various diseases. Phytochemical screening of extracts revealed the presence of various metabolites. The obtained scientific data may be beneficial for development of formulations, biochemistry of natural products, molecular development and preparing the monograph of plant.

ACKNOWLEDGEMENT

A hearty thanks to Dr. Narendra Kumar Singh, Associate Professor, BBD University for their valuable suggestions and support.

CONFLICT OF INTEREST

This authors declare no conflict of interest.

ABBREVIATIONS

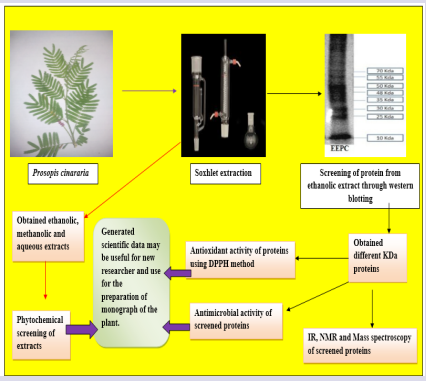
P. cineraria, *Prosopis cineraria;* **DPPH:** 1, 1-diphenyl-2- picryl - hydrazyl; ***S. aureus:*** *Staphylococcus aureus*, ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***A.tubingensis:*** *Aspergillus tubingensis;* **AEPC:** Aqueous extract of *P. cineraria*; **ZOIs:** Zone of Inhibitions.

REFERENCES

1. Firenzuoli F, Gori L, Crupi A, Neri D. Flavonoids: risks or therapeutic opportunities?. Recenti Prog Med. 2004;95(7-8):345-51.
2. McGoverin CM, Weeranantanaphan J, Downey G, Manley M. The application of near infrared spectroscopy to the measurement of bioactive compounds in food commodities. J Near Infrared Spectrosc. 2010;18(2):87-111.
3. Lu X, Rasco BA. Determination of antioxidant content and antioxidant activity in foods using infrared spectroscopy and chemometrics: a review. Crit Rev Food Sci Nutr. 2012;52(10):853-75.
4. Agrawala VS. Studies in Indian Art, ed. Varanasi: Vishwavidyalaya Prakashan.1965;111-32.
5. Shubhangi PD, Patil A. Ethnobotany of Jalgaon District, Maharashtra. 3rd ed. New-Delhi: Daya Publication House. 2008;555.
6. Puri S, Kumar A. Establishment of *Prosopis cineraria* (L.) Druce in the hot deserts of India. New Forest. 1995;9(1):21-33.
7. Joshi SG. Medicinal Plants. 1st ed. Delhi: Published by Mohan Primlani for Oxford and IBH Publishing Co. Pvt. Ltd. 2002;141.
8. Malik A, Kalidhar SB. Phytochemical examination of *Prosopis cineraria* L. (Druce) leaves. Indian J Pharm Sci. 2007;4:576-8.
9. Jewers K, Nagler MJ, Zirvi KA, Amir F. Lipids, sterols and a piperidine alkaloid from *Prosopis spicigera* leaves. Phytochem. 1976;15(1):238-40.
10. Panda S, Jafri M, Kar A, Meheta BA. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia. 2009;80(2):123-6.
11. Rastogi RP, Malhotra BN. Compendium of Indian Medicinal Plants, Lucknow/ New Delhi (Central Drug Research Institute (India), Council of Scientific and Industrial Research (India). Publications and Information Directorate), 1995;4.
12. Ghazanfar SA, Alsabahi AMA. Medicinal plants of Northern and Central Oman (Arabia). Econ Bot. 1993;47(1):89-98.
13. Sharma H, Kumar A. Ethanobotanical studies on medicinal plants of Rajasthan (India). J Med Plants Res. 2011;5(7):1107-12.
14. Nagori BP, Solanki R. Role of medicinal plants in wound healing. J Med Plants Res. 2011;5(4):392-405.
15. Ramasamy VM, Venugopalan R, Ramnathan SK, Perumal P, Chellapan DR. Analgesic and antipyretic activity of stem bark of *Prosopis cineraria* (Linn) Druce. J

Pharm Res. 2009;2(4):660-2.
16. Joseph L, George M, Sharma A, Gopal N. Antipyretic and analgesic effects of the aqueous extracts of *Prosopis cineraria*. Global J Pharmacol. 2011;5(2):73-7.
17. Sharma N, Garg V, Paul A. Antihyperglycemic, antihyperlipidemic and Antioxidative Potential of *Prosopis cineraria* bark. Indian J Clin Biochem. 2010;25(2):193-200.
18. Purohit HR. Hypolipidemic and anti-atherosclerotic effects of *Prosopis cineraria* bark extract in experimentally induced hyperlipidemic rabbits. Asian J Pharm and Clin Res. 2012;5(3):106-9.
19. Purohit HR. Effect of *Prosopis cineraria* bark extract on Hematology in hypercholesterolemic rabbits. Indian J Fundam and Appl Life Sci. 2012;2:96-100.
20. Robertson S, Narayanan N, Kapoor BR. Antitumour activity of *Prosopis cineraria* (L.) Druce against Ehrlich ascites carcinoma-induced mice. Nat Prod Res. 2011;25(8):857-62.
21. Maideen NMP, Velayutham R, Manavalan G. Protective activity of *Prosopis cineraria* against N-Nitrosodiethylamine induced liver tumours in accordance to mitochondrial lipid peroxidation, mitochondrial antioxidant and liver weight. Continental J Pharm Sc. 2011;5(2):1-6.
22. Bithu BS, Reddy NR, Prasad SR, Sairam K, Hemalatha, S. *Prosopis cineraria*: a potential nootropic agent. Pharm Biol. 2012;50(10):1241-7.
23. Janbaz KH, Haider S, Imran I, Zia-Ul-Haq M, Martino L, Feo VD. Pharmacological evaluation of *Prosopis cineraria* (L.) Druce in gastrointestinal, respiratory and vascular disorders. JEBCAM. 2012;3:1-7.
24. Velmurugan V, Arunachalam G, Ravichandran V. Anticonvulsant activity of methanolic extract of *Prosopis cineraria* (Linn) Druce stems barks. Int J Pharm Tech Res. 2012;4(1):89-92.
25. Velmurugan V, Arunachalam G, Ravichandran V. Antibacterial activity of stem barks of *Prosopis cineraria* (Linn.) Druce. Archives of Appl Sci Res. 2010;2(4):147-50.
26. Salar RJ, Dhall A. Antimicrobial and free radical scavenging activity of extracts of some Indian medicinal plants. J Med Plants Res. 2010;4(22):2313-20.
27. Napar AA, Bux H, Zia MA, Ahmad MZ, Iqbal A, Roomi S. Antimicrobial and antioxidant activities of Mimosaceae plants; *Acacia modesta* Wall (Phulai), *Prosopis cineraria* (Linn.) and *Prosopis juliflora* (Swartz). J Med Plants Res. 2012;6(15):2962-70.
28. Robertson S, Narayanan N, Ravinargis NR. Toxicity evaluation on hydroalcoholic extracts of leaf and stem bark of *Prosopis cineraria*. Int J Pharm and Pharma Sci. 2012;4(3):113-8.
29. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 35th ed. Nirali Prakashan. Pune. 2006;102.
30. Kulshreshtha M, Singh MP. Pharmacognostic Standardization and HPTLC Fingerprinting of *Prosopis cineraria*; An Ayurveda Mentioned Plant. Pharmacogn Commn. 2019;9(1).
31. Kulshreshtha M, Dwivedi H, Singh MP. Antimicrobial effects of leaves of Indian herbal plants with reference to peptic ulcer. Environ Dis. 2018;3(1):18-26.
32. Jyoti G, Mayank K. Pharmacological Investigation and Unravelling Mechanism of Action of *Jasminum sambac* Flowers for Predicated Treatment of Alzhemer's Disease. Curr Nutr Food Sci. 2017;13:1-8.
33. Mayank K, Gunja S, Manjul PS. Pharmacognostical, Anti-oxidant activity and High-Performance Thin Layer Chromatography studies on leaves of *Quisqualis indica* Linn. CTM. 2018;4(1):53-67.
34. Shukla KS, Chawla P, Pandey S. Synthesis, characterization and screening of thiazolidine-2,4-dione derivatives as antimicrobial agents. Int J Microbiol Res. 2016;8:807-12.
35. Verma S, Gupta R. Pharmacognostical and high-performance thin lawyer chromatography studies on leaves of *Clerodendrum infortunatum* L. AYU. 2014;35(4):416-21.
36. Hoffman RJ, Michael JF. Protein–Which is Best?. J Sports Sci Med. 2004;3(3):118-30.
37. Knezevic SV, Blazekovic B, Stefan MB, Alegro A, Koszeg T, Petrik J. Antioxidant activities and polyphenolic contents of three selected Micromeria species from Croatia. Molecules. 2011;16(2):1454-70.
38. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish J Environ Stud. 2006;15(4):523-30.

PICTORIAL ABSTRACT



SUMMARY

- Proteins are necessary and important nutrient for human body. Sources of protein include plants, dairy products, meat, eggs, etc. In the present research, the various proteins have screened out from ethanolic extract of *Prosopis cineraria* leaves. The obtained proteins have different KDa weight with good antioxidant and antimicrobial potential. The generated scientific data may act as a backbone of various new plants based researches and give the new fruitful ideas to new scientists in near future. This paper is the second important part of my Ph.D research. The first part is published on same journal (Pharmacognosy Communications) under the title "Pharmacognostic Standardization and HPTLC Fingerprinting of *Prosopis cineraria*; An Ayurveda Mentioned Plant".

ABOUT AUTHORS

Mr. Mayank Kulshreshtha, has completed his M. Pharm in Pharmacology and Ph.D in Pharmaceutical Sciences on the topic "PHARMACOLOGICAL STUDIES ON SELECTED INDIAN MEDICINAL PLANTS". He is working as an Assistant Professor in Department of Pharmacology, School of Pharmacy, Babu Banarasi Das University, Lucknow (U.P.), India. He is life time member of various pharmaceutical and medical associations. He is also a reviewer of various national and International journals.

Dr. Manjul Pratap Singh, working as an Associate Professor in School of Pharmacy, Babu Banarasi Das University, Lucknow (U.P.), India. He has guided lots of Ph. D students and a life time member of various pharmaceutical associations.