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SCREENING AND PURIFICATION OF ANTIBACTERIAL PROTEINS AND PEPTIDES FROM SOME OF THE MEDICINAL PLANTS SEEDS

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ABSTRACT

Antibiotics have been effective in treating infectious diseases, but resistance to these drugs has led to the emergence of new and the reemergence of old infectious diseases. For centuries, Indian spices have made a significant contribution both in the health care system and the food industry. Ancient Asian literature is a treasure of information related to the problems of health care and other environmental aspects. Bacterial strains used for the study was *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *P. vulgaris* (ATCC 6380) were purchased from Hi-Media laboratories. Our results showed that various buffer pH have different protein extractability percentages after dialysis for the seed extracts used. The protein was extracted by sodium phosphate citrate buffer pH (7.2) highest concentration of the protein was found to (660µg/ml) in *Ammi majus* and the *lowest* was 160 µg/ml in . The highest protein concentration extracted by CTAB buffer pH (6.0) was found to be (640µg/ml) in *Ammi majus* and the lowest was 140 µg/ml in *Chichorium intybus.*

KEYWORDS: ***Medicinal plants, Antimicrobial Activity, Bacterial strains.***

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INTRODUCTION

For centuries, Indian spices have made a significant contribution both in the health care system and the food industry. Ancient Asian literature is a treasure of information related to the problems of health care and other environmental aspects. Indian spices have been used since ages in different traditional forms of medicine like Ayurveda, Unani and Sino Tibetian systems. In an era characterized by increasing consumer choice, self-medication and quest for natural therapy, herbal products are used increasingly as an alternative to drugs and supplements[1]. In particular, extracts from many kinds of oriental spice plants are known to possess antimicrobial effect besides being used for the purpose of food preservation, appetiser promotion and medicinal purposes[2-4]. Today, the exploration of naturally occurring antimicrobials for food preservation has received increasing attention. This is attributed to the consumer awareness of natural food products and a growing concern for microbial resistance towards conventional preservation[5]. An interrelationship between the health-benefiting properties of spices and their use in food needs to be scientifically re-established. Contamination of food caused by unsanitary practices compromises the health of people at various levels. Food safety, hence, becomes a key concern for the food-processing industry. In addition to these hygiene practices, several food preparation techniques, such as preservation in spices have been used for thousands of years. The use of spices in traditional cooking is well known, and the antimicrobial effect of spices, such as turmeric is well documented[6]. Infectious diseases are caused by bacteria, viruses, parasites and fungi, and it is due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics had eradicated the infections that once ravaged the humankind. But their indiscriminate use has led to the development of multidrug-resistant pathogens[7]. Around 90–95% of *Staphylococcus aureus* strains worldwide are resistant to penicillin[8] and in most of the Asian countries 70–80% of the same strains are methicillin resistant[9]. Our Previous study on Evaluation of antibacterial activity of crude protein extracts from seed shown an immense results against standard bacterial strains. These plants can be used to discover bioactive natural products in the form of antimicrobial proteins and peptides that may serve for the development of new pharmaceuticals. Such screening of various natural antimicrobial proteins and peptides supports the traditional knowledge of local users and it is a preliminary scientific validation for the use of these plants for the antibacterial activity. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings[10]. Present study was focused on the Screening and purification of antibacterial proteins and peptides from the seeds of *Chichorium intybus, Hyoscyamus niger* and *Ammi majus* were tested against bacterial pathogens namely *E. coli, S. aureus, P. aeruginosa* and *P. vulgaris.*

MATERIALS AND METHODS

MEDICINAL PLANTS

The medicinal plant seeds were collected from Pharmacy of Central Research Institute of Unani Medicine, Hyderabad.

PREPARATION OF PLANT SEED EXTRACTS

All the plants seeds were first cleaned using tap water in order to remove any dirt or debris, and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. And were grinded using grinder to make fine powder. Antimicrobial proteins and peptides were extracted using sodium phosphate citrate buffer (pH 7.2) and sodium acetate buffer (pH 6.5). The buffers were prepared and seeds of these medicinal plants were incubated at 28–30 _C and ground in these buffers and the extract was filtered using Whattmann filter paper No. 1. The crude extract isolated was saturated with 80% ammonium sulfate. The saturated extract was subjected for dialysis. After dialysis these samples were subjected to spectrophotometric analysis.

PROTEIN DETERMINATION

Protein concentrations were determined as described by lowry method[11]. Briefly, an aliquot of appropriate dilution of sample (10-50 µL) was mixed with 200 µL of commercial protein determination reagent (Bio-Rad), and made up to 1 mL with distilled water. After mixing and stand in room temperature for 5 min, the absorbance was read at 595 nm, and the protein content was calculated with a BSA as standard.

BACTERIAL STRAINS

Bacterial strains used for the study was *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *P. vulgaris* (ATCC 6380) were purchased from Hi-Media laboratories.

CULTURE MEDIUM AND INOCULUM PREPARATION

High sensitivity testing agar (Hi-Media) was used for checking antibacterial activity of crude protein extracts of different plant seeds against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *P. vulgaris* (ATCC 6380). The microbial strains were cultured on the slants in the sterilized Laminar Air Flow from the pure culture. These cultured slants were incubated at 37 ^{0}C for bacterial growth for 2–3 days. High sensitivity testing agar was mixed at a concentration of 23.4 g/1000 ml in distilled water and autoclaved at 121 _C for 15 min. A loop full from pure culture of a bacterial strain was mixed in the 10 ml of Nutrient broth medium[10]. And incubated at 37 ^{0}C overnight The final concentration of the inoculum for the four bacterial strains used in the experiment was 108 CFU/mL). For every experiment, freshly prepared sterile nutrient broth (10 mL) was inoculated from the slants and the activated culture was used for streaking onto the agar plates for antimicrobial sensitivity.

AGAR WELL DIFFUSION ASSAY

The antibacterial activity of the crude protein extracts was determined by Agar well diffusion assay[12]. 2.34 g of high sensitivity testing agar was dissolved in 100 ml of distilled water and autoclaved at 121 ^{0}C for 15 min. Before transferring this medium in sterilized petri plates, it was allowed to cool and then was poured into the petri plates and allowed to solidify. After this, it is inoculated with activated culture using sterile cotton swabs. And the wells are created using sterile agar borer and the wells were filled by adding 100µL of crude protein extracts and were incubated at 37 ^{0}C for 12–24 h. Three replicates were prepared from each sample. The extracts having antimicrobial activity, inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters. All the analyses were applied in triplicates[13].

PURIFICATION OF PROTEIN USING DIALYSIS TECHNIQUE

Antimicrobial proteins and peptides were extracted using Sodium phosphate citrate buffer (pH7.2) CTAB buffer (pH 6) Sodium acetate buffer (pH 6.5). The seeds of these medicinal plants were incubated at 28-30 ^{0}C and grinded in these buffers and the extract was filtered using Whattmann filter paper No. 1. The crude extract isolated was saturated with 80% ammonium sulphate. The saturated extract was subjected for dialysis using 3kDa Dialysis tubing (Sigma)[14].

PURIFICATION OF PROTEINS USING ION-EXCHANGE CHROMATOGRAPHY

After Dialysis the sample was subjected for ion exchange chromatography using 0.3% DEAE cellulose column using Sodium Phosphate Citrate Buffer (Ph-7.2) and Elutes were run for 4hrs and collected 2ml each. The antibacterial activity of the purified protein extracts was determined by Agar well diffusion assay. Nutrient agar was used for the assay. The protein extracts having antibacterial activity, inhibits the bacterial growth and the clear zones were formed. The zone of inhibition was measured in millimeters.

RESULTS AND DISCUSSION

Numerous types of molecules with antibacterial activity have been isolated from plants[15,16]. Among them proteins and peptides with antimicrobial activity have recently been reported. They are recognized as important

components of the innate defense system of bacteria, fungi, insects, animals and plants. Most of these defense proteins and peptides normally have multitasked activities. Some peptides can selectively inhibit gram positive or negative bacteria although antimicrobial peptides with gram positive and gram negative bacteria growth inhibiting ability have been reported[17].

CRUDE PROTEIN CONCENTRATION OF DIALYSIS SAMPLES

Our results showed that various buffer pH have different protein extractability percentages after dialysis for the seed extracts used (Table 1). The protein was extracted by sodium phosphate citrate buffer pH (7.2) highest concentration of the protein was found to (660mcg/ml) in *Ammi majus* and the *lowest* was 160 µg/ml in . The highest protein concentration extracted by CTAB buffer pH (6.0) was found to be (640µg/ml) in *Ammi majus* and the lowest was 140 µg/ml in *Chichorium intybus*. Amount of protein concentration was found to be different in comparison with various buffers extraction[18].

Table 1
Comparison of protein concentration (µg/mL) in plant extract by different buffers after dialysis.

S.no	Plants	Sodium Phosphate Citrate Buffer(pH 7) [**µg/mL]**	CTAB Buffer (pH 6.0) [**µg/mL]**
1.	*Chichorium intybus L.*	160	140
2.	*Hyoscyamus niger L.*	280	340
3.	*Ammi majus L.*	660	640

ANTIBACTERIAL ACTIVITY OF CRUDE PROTEIN EXTRACTS AFTER DIALYSIS OBTAINED FROM DIFFERENT PLANT SPECIES

Antibacterial activity of protein and peptides after dialysis from different plant seed purified in various buffers were tested against four different bacterial strains which were studied. Antibacterial activity of purified proteins and peptides extracted in sodium phosphate citrate buffer pH (7.2) subjected for dialysis among all *chichorium intybus* and *AmmiMajus* was found to be very effective on *Proteus vulgaris* with a zone of inhibition 25mm and 20mm. The extract prepared in CTAB buffer (pH-6) of different plant seeds found to shown good activity on *Proteus vulgaris* for *Chichorium intybus* with a zone of inhibition 28mm. *Ammi majus* was found to shown very good activity on E.coli with zone of inhibition diameter 27mm and was less effective on *Staphylococcus aureus* and *Proteus vulgaris* with a zone of inhibition diameter 2mm. Most of the strains found to have shown better sensitivity compared with the standard antibiotics Chloramphenicol (25 mcg) and Ciprofloxacin (100mcg) (Table 2).

Table 2
Represents the results of Dialysis samples antimicrobial activity test (Diameter of Zone of Inhibition) (mm)

S.No		Sodium Phosphate Citrate Buffer pH 7.2 [A]			CTAB Buffer pH 6.0 [B]				
		S 1	S 2	S 3	S 1	S 2	S 3	Chloramphenicol (25mcg)	Ciprofloxacin (100mcg)
1	*Staphylococcus aureus*	13	14	08	14	14	02	21	16
2	*E coli*	13	15	11	18	14	27	08	14
3	*Pseudomonas aeroginosa*	02	14	0	18	0	13	08	12
4	*Proteus vulgaris*	25	20	11	28	0	02	08	14

Chichorium intybus L(S1), Hyoscyamus niger L(S2) and Ammi majus L(S3).

PROTEIN CONCENTRATION OF ION EXCHANGE CHROMATOGRAPHY ELUTES

The crude protein extract after Dialysis was subjected for ion exchange chromatography and four elutes were obtained and the concentration was found out using the lowry method. The concentration of the protein was found abundant in *Ammi majus* seed extract in all the elutes ranges from 560 -600 (mcg/ml). And the least concentration was found in *Chichorium intybus* ranges from 100-140 (mcg/ml) and the moderate concentration was obtained in *Hyoscyamus niger* ranges from 200-250 (mcg/ml) (Table 3).

Table 3
Represents the protein concentration of ion exchange chromatography elutes

S.No	Plants	Concentration of protein after Ion-exchange chromatography (mcg/ml).			
		Elute 1	Elute 2	Elute 3	Elute 4
1.	*Chichorium intybus L.*	140	140	110	100
2.	*Hyoscyamus niger L.*	250	220	230	200
3.	*Ammi majus L.*	600	580	580	560

ANTIBACTERIAL ACTIVITY OF ION EXCHANGE CHROMATOGRAPHY ELUTES

The crude plant extracts after dialysis was subjected to run ion exchange chromatography and the antibacterial activity of four elutes were performed on selected bacterial strains. Among all the elutes of *Chichorium intybus* elute 2 was found to be very effective on *E.coli* with Diameter of zone of inhibition 23mm with IC50 value 62.64 (mcg/ml) and *Ammi majus* shown good activity on *Staphylococcus aureus* with zone of inhibition 23mm and IC50 value 324.60 (mcg/ml). Elute 4 of *Hyoscyamus niger* shown highest activity on *Proteus vulgaris* with 22 mm diameter of zone of inhibition and IC50 value 102.77(mcg/ml). All the four elutes of different plants used for the study reveals lowest to moderate activity on *Pseudomonas aeruginosa* strain with a diameter of zone of inhibition ranges from 05 to 16mm (Table 4) with IC50 value ranges from 25.20 to 638.48(mcg/ml) (Table 5).

Table 4
Represents the results of Ion Exchange Chromatography Elutes Antimicrobial activity (Diameter of zone of inhibition[mm])

Hyoscyamus niger L	**Elute 1**	**Elute 2**	**Elute 3**	**Elute 4**
Staphylococcus aureus	13	13	16	14
E coli	12	14	15	15
Pseudomonas aeruginosa	11	16	16	14
Proteus vulgaris	12	15	18	22
Chichorium intybus L				
Staphylococcus aureus	13	13	16	14
E coli	16	23	13	19
Pseudomonas aeruginosa	11	07	09	05
Proteus vulgaris	11	11	16	13
Ammi majus L				
Staphylococcus aureus	16	23	13	19
E coli	12	14	15	15
Pseudomonas aeruginosa	15	13	16	16
Proteus vulgaris	17	15	12	17

Table 5
Represents the IC 50 values of Ion Exchange Chromatography Elutes.

Hyoscyamus niger L	**Elute 1**	**Elute 2**	**Elute 3**	**Elute 4**
Staphylococcus aureus	47.65	54.29	63.20	37.20
E coli	49.01	116.18	124.86	110.18
Pseudomonas aeruginosa	115.69	121.46	42.56	38.21
Proteus vulgaris	115.69	108.65	116.04	102.77
Chichorium intybus L				
Staphylococcus aureus	29.16	19.97	25.60	25.20
E coli	58.38	62.64	47.36	41.96
Pseudomonas aeruginosa	66.18	79.29	36.93	30.09
Proteus vulgaris	56.84	37.34	48.94	45.66
Ammi majus L				
Staphylococcus aureus	297.61	324.60	106.83	105.05
E coli	705.55	207.26	214.05	182.67
Pseudomonas aeruginosa	107.75	113.19	110.434	110.20
Proteus vulgaris	616.65	638.48	218.90	214.23

CONCLUSION

This study supports the traditional knowledge of local users and it is a preliminary scientific validation for the use of these plants for the antibacterial activity. This research implies the possible application of crude proteins extracts in foods and pharmaceuticals.

ABBREVIATION

ATCC = American type culture collection
BSA = Bovine serum albumin
CFU = Colony forming unit
DEAE= diethylaminoethyl
IC_{50} = Half maximal inhibitory concentration
CTAB = Cetyltrimethyl ammonium bromide

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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