Journal of Medicinal Plants Research Vol. 6(4), pp. 566-573, 30 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.613
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Isolation and characterization of antimicrobial activity conferring component(s) from seeds of bitter gourd (*Momordica charantia*)

Abid Mahmood[1], Ghazala Kaukab Raja[1], Tariq Mahmood[2]*, Muhammad Gulfraz[1] and Azra Khanum[1]

[1]Department of Biochemistry, University of Arid Agriculture Rawalpindi, Pakistan.
[2]Department of Plant Sciences, Quaid-i-Azam University, Islamabad-45320, Pakistan.

Accepted 22 November, 2011

***Momordica charantia*, a commonly used vegetable in Pakistan, has been known for its medicinal importance to cure diabetes but its antimicrobial activity has not been explored yet. Present study was designed to investigate antimicrobial activity of *M. charantia* seed extracts against mammalian and poultry pathogens. Buffered saline, ethanol, n-hexane, petroleum ether based seed extracts of *M. charantia* were prepared to screen anti-microbial activity by disc diffusion method. The aqueous seed extract showed highest antimicrobial activity against *Pasteurella multocida* while ethanol, n-hexane and petroleum ether extracts were effective against *Staphylococcus aureus.* The protein/peptides were isolated by 80% ammonium sulphate precipitation and gel filtration chromatographyand separated on SDS-PAGE. The electrophoretic profile of dialyzed seed extracts showed seven bands of peptide/protein bands ranging from ~9 to ~54 kDa. The protein/peptides seed extract was efficient against *S. aureus* and *Salmonella typhi* with the maximum zone of inhibition (1 cm). All results were compared with chloramphenicol, an antibiotic with highest antimicrobial activity against all tested microorganisms.**

Key words: Bitter gourd, antimicrobial peptides, chromatography.

INTRODUCTION

Application of medicinal plants and their products for the treatment of various human ailments had been made by man since ancient time and authentic documented record for the application of medicinal plants is available in Rigveda (3500 to 1600 B.C.) and Atharva Veda (1200 B.C) (Parasad et al., 2002). Many researchers have worked towards the medicinal strength of different parts of some higher plants and their chemical compounds (Onyeagba et al., 2004) like; anthelmintic, antibacterial, antibiotic, antidiabetic, anti-inflammatory, anti microbial, antileukemic, antimutagenic, antimycobacterial, antioxidant, antitumor, antiulcer, antiviral, aperitive, aphrodisiac, astringent, carminative, cytostatic, cytotoxic, depurative, hormonal, hypocholesterolemic, hypotensive, hypotriglyceridemic, hypoglycemic, immunostimulant, insecticidal, lactagogue, laxative, purgative, refrigerant, stomachic, styptic, tonic, vermifuge and antifungal (Adekunle and Okoli, 2002). Pakistan is one of the few places on earth which are blessed with a unique biodiversity, comprising of different climatic zones with a wide range of plant species. Approximately 15,000 plant species with medicinal properties have been discovered and used in herbal medicines but majority of the herbal medicine extracts are imported from other parts of the world. In addition, chemical/biochemical characterization of various plant components and medicinal properties has not been studied in detail.

Thus, there is a dire need on the part of local allopathic and herbal medicine manufacturers to carryout systematic research on medicinal plants to save foreign exchange spend on their imports (Shinwari, 1989). *Momordica charantia, a* member of Cucurbitaceae family, is a tropical plant currently distributed across the globe. *M. charantia,* commonly known as bitter gourd or bitter

*Corresponding author. E-mail: tmahmood@qau.edu.pk.

melon, is a monoecious climber with oblong, green colored fruit that is extensively ribbed. Some of the major constituents of *M. charantia* L. are alkaloids, charantin, charine, goya glycosides, goyasaponins, lenoleic and lenolinic acid, other acids are, peptides and proteins (Kight, 2003).

The fruit is used both as a food item and a wide-ranging medical material in many traditional medical systems. Because of its widespread use and economic importance, the *M. charantia* has long been a focus research at various scientific institutions around the globe. Several water, ethanol, and methanol based extracts of the bitter melon leaves have demonstrated *in vitro* antibacterial activities against *Escherichia coli,* Staphylococcus, Pseudomonas, Salmonella, Streptobacillus and Streptococcus. While an extract of the entire plant was shown to have antiprotozoal activity against *Entamoeba histolytica* (Khan et al., 1998).

Besides being a rich source of antimicrobial natural compounds*, M. charantia* has also been documented with antiviral activity against Epstein Barr, Herpes and HIV viruses. In an *in vivo* study, a leaf extract of plant demonstrated the ability to increase resistance against common viral infections of humans and animals by increasing interferon production and natural killer cell activity as well as providing an immune-stimulant effect. Two proteins known as alpha- and beta-momorcharin (present in the seeds, fruit, and leaves of *M. charantia*) have been reported to inhibit HIV virus growth *in vitro* (Ng et al., 1992).

The fruit and leave phenolic compounds extracts of *M. charantia* are being studied and used extensively for their medicinal properties. However, major peptide/proteins fractions in *M. charantia* still need to be identified and characterized especially those of seeds which normally are being discarded. By keeping in view the natural richness of *M. charantia* with its useful organic compounds, the present study was designed to isolate and characterize principally active component(s) from the seeds of *M. charantia* and to evaluate their antimicrobial properties against some common mammalian and poultry diseases causing microbes.

MATERIALS AND METHODS

Collection of vegetable

M. charantia L. was collected from the local markets of Rawalpindi city, Pakistan. The vegetable was brought to the laboratory and rinsed with water, surface sterilized and refrigerated till further analysis.

Preparation of aqueous seed extract

The seeds of *M. charantia* were separated from the fruits, dried and ground to fine powder in mortar and pestle. To make aqueous extract, 10 g of seed powder was blended in 150 ml of normal saline buffer (PBS), pH 7.2. The seed extract was frozen and thawed thrice, followed by centrifugation at 10,000 rpm at 4°C for 10 min.

The aqueous extract of seeds was then filtered through a sterile Watman filter paper. The supernatant was then stored for aqueous extract and pellet was discarded.

Preparation of organic seed extract

To prepare organic seed extract, 10 g of the powdered samples were separately soaked in 50 ml of 70% ethanol, petroleum ether and n-hexane for 24 h. After 24 h, the three seed extracts were filtered through common Watman filter paper and centrifuged at 10,000 rpm at 4°C for 10 min. The supernatants were collected and dried in a rotatory evaporator at 30°C. The pellets obtained were dissolved in distilled water and stored at 4°C till further use.

Peptide/protein extraction from aqueous seed extract

For peptide/protein extraction, from *M. charantia* crude aqueous seed extracts was performed using ammonium sulphate precipitation at 80% salt solution followed by centrifugation at 10,000 rpm for an hour at 25°C. The clear supernatant and pellet were dissolved in deionized water and dialyzed (using MWCO 3500 Da membrane Spectra/Por® cat # 132721), overnight at room temperature, against distilled water.

Protein estimation using bradford assay

Total protein content of the *M. charantia* seed extracts was estimated spectrophotometrically by Bradford method using commercial kit method against BSA stock solution (2 mg/ml) at 595 nm.

Antimicrobial activity determination

For the determination of antimicrobial activity of various *M. charantia* seed extracts, the following steps were carried out;

Preparation of bacterial stocks

Cultures of bacteria; against ten bacterial pathogens namely *E. coli*, *Staphylococcus aureus* (*S. aureus*), *Salmonella typhimurium* (*S. typhi*), *Pasteurella multocida* (*P. multocida*.), *Lactobacillus bulgaricus* (*L. bulgaricus*), *Micrococcus luteus* (*M. luteus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Proteus vulgaris* (*P. vulgaris*), were obtained as glycerol stocks already maintained at -80°C from Depository of Biotechnology Laboratory, PMAS Arid Agriculture University Rawalpindi. All selected cultures were revived to be used in present study.

Fresh glycerol stocks were prepared by inoculating old bacterial stocks using streaking method on Lauria Bertaini (LB) agar plates (appendix I) and plates incubated overnight at 37°C. The starter culture was prepared by mixing isolated bacterial colonies with 3 ml of LB broth incubated at 37°C for overnight in shaker incubator. This culture was used to prepare glycerol stocks.

Then the optimal densities (OD) of cultures were measured at 600 nm. A culture with a 0.4 OD_{600nm} (10^8 CFU/ml) is selected for glycerol stocks and finally stored at -80°C for further analysis.

Zone inhibition assay

To determine antimicrobial activity by the disc diffusion method

(Adekunle and Okoli, 2002), respective cultures were applied on individual nutrient agar plates under sterilized conditions. Sterilized paper discs (Whatman filter paper discs of 6.0 mm in diameter) were soaked in various seed extracts of *M. charantia* was and placed on nutrient agar cultures. As control, disc soaked in antibiotic chloramphenicol was applied on each plate at separate compartment and all tests plates along with control were incubated at 37°C for 16 h. Positive results of zone inhibition assay were measured by the size of the clear zones around the discs area as compared to a control; antibiotic Chloramphenicol. The size of cleared zones was measured in cm using a ruler and plates were photographed. Clear zones ranging between 0.8 to 1 cm diameters were regarded to possess higher level of antimicrobial activity. All assays were conducted in triplicate and overall activity of seeds was taken as average.

Gel filtration chromatography

Gel filtration chromatography was carried out using Sephadex G-100 (Bed volume 15 to 20 ml/g dry Sephadex 1.5 x 70 cm long, flow rate 1 ml/min). A 2 ml dialyzed seed extract (683 µg /ml protein content) was applied on top of column and a total of 30 fractions (5ml each) were collected. The eluted samples were checked for the presence of protein/peptide spectrophotometrically at an absorbance of 280 nm and results were plotted on a graph. Peaks fractions with highest absorbance were lyophilized and analyzed using 12% SDS-PAGE (Sambrook and Russell, 2001) along with pre-stained protein marker (Fermentas® cat # SM 0431).

Statistical analysis

Antimicrobial activity of different *M. charantia* seed extracts using zone inhibition assay was measured by mean ± SD. The results were expressed in cm as a clear zone and were analyzed by two factor ANOVA (followed by a Fisher PLSD a *posteriori* test, $p<0.05$) using SPSS.

RESULTS AND DISCUSSION

Present study was focused on the isolation and characterization of antimicrobial activity conferring components from the seeds of *M. charantia* L. For antimicrobial activity, aqueous, ethanol, petroleum ether and n-hexane extracts of *M. charantia* seed were prepared and tested against ten bacterial pathogens namely *E. coli*, *S. aureus, S. typhi*, *P. multocida*, *L. bulgaricus*, *M. luteus*, *P. aeruginosa*, *K. pneumoniae*, *S. epidermidis* and *P. vulgaris*. The ammonium sulphate precipitated proteins/peptides fraction (80%) and supernatant were also checked for their antimicrobial activity against the above listed bacteria. Protein content of crude aqueous extract of seeds was 625 µg/ml while the dialyzed pellet and supernatant of seed extract were 683 and 379 µg/ml, respectively. These results are in accordance with Kight (2003) who reported peptides and proteins along with alkaloids, charantin, charine, goya glycosides, goyasaponins, lenoleic and lenolinic acid, other acids, as major constituents of *M. charantia* L.

Seed extracts and purified peptides had shown antimicrobial activity against selected bacteria in varying order as compared to the standard antibiotic that is, chloramphenicol which has shown the activity against all ten bacterial strains used in this experiment (Table 1 and Plate 1). The aqueous seed extract produced significant antimicrobial activity against *P. multocida* (0.7 ± 0.06), *S. epidermidis* (0.2 ± 0.03), *S. typhi* (0.3 ± 0.05) and *L. bulgaricus* (0.2 ± 0.03). The ethanol seed extract showed significant antimicrobial activity against *S. aureus* (0.6 ± 0.05), *M. luteus* (0.5 ± 0.01), *E. coli* (0.4 ± 0.06), *S. epidermidis* (0.4 ± 0.05) and *L. bulgaricus* (0.2 ± 0.05). While n-hexane and petroleum ether extracts were effective against *S. aureus* (0.4 ± 0.06), (0.6 ± 0.05), respectively. Present findings are in accordance with previous studies conducted to study antimicrobial activities of water, ethanol, and methanol based extracts of the bitter melon leaves against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella, Streptobacillus* and *Streptococcus* (Dulger and Gonuz, 2004).

Antimicrobial activity of dialyzed ammonium sulphate pellet and supernatant of the *M. charantia* L. seed was also checked on LB nutrient agar plates against ten bacterial strains (Plate 2). Results clearly indicate (Table 2) that dialyzed ammonium sulphate pellet and supernatant have significant antimicrobial activity against *E. coli* (0.6. ± 0.05), (0.6 ± 0.05), *S. aureus* (0.7 ± 0.03), (0.7 ± 0.03), *S. epidermidis* (0.3 ± 0.05), (0.4± 0.05), *S. typhi* and *L. bulgaricus* (0.6 ± 0.05), (0.2 ± 0.03) respectively. Studies carried out by Anwar (2000) and Shetty (2005) on Arbuscular mycorrhizal roots of *Allium sativum* L. and fruit of bitter gourd respectively also report significant antimicrobial activity associated with the plant extracts.

The chromatographic profile of ammonium sulphate precipitated and dialyzed *M. charantia* seed protein/peptide fractions (Figure 1) clearly show a single significant peak. The findings are consistent with studies of Ng et al. (1992) where peptides, called alpha- and beta-momorcharins, were isolated from seeds of *M. charantia* through aqueous extraction, acetone fractionation and ion exchange chromatography. The molecular weight profiling of the crude aqueous extract and dialyzed seed extract consisted of crude extract of *M. charantia* seed using 12% SDS-PAGE is shown in Figure 2, clearly indicates presence of several proteins with the molecular weight ranging from ~10 – 55 kDa as already reported in previous studies (Parkash et al. 2002, Sheng et al., 2004). However, in present study, sequencing and characterization of the isolated peptides/proteins was not performed. The peak fraction, collected from gel filtration column, was then tested for antimicrobial activity on LB nutrient agar plates against ten bacterial strains (Plates 3 and Table 2). The peak fraction showed significant antimicrobial activity only against *E. coli* (0.8 ± 0.02), *S. aureus* (1.0 ± 0.09), *S. typhi* (1.0 ± 0.02), *Klebsella pneumoniae* (0.8 ± 0.02) and *Micrococcus luteus* (1.0 ± 0.09). Present results come in accordance with Rasheed et al. (1990) where antimicrobial activity of Sephadex

Table 1. Antimicrobial activity of aqueous and organic seed extracts of *Momordic acharantia L* checked against pathogenic bacteria.

Bacteria	Aqueous seed extract (cm)	Control (cm)	Ethanol seed extract (cm)	Control (cm)	n-hexane seed extract (cm)	Control (cm)	Petroleum ether seed extract cm	Control cm
E. coli	0.00	0.3±0.02	0.4±0.06	1.1±0.01	0.00	1.1±0.01	0.00	0.9±0.03
K. pneumonia	0.1±0.03	0.7±0.01	0.00	0.9±0.02	0.00	0.8±0.02	0.1±0.03	0.7±0.01
L. bulgaricus	0.2±0.03	0.7±0.01	0.2±0.05	0.8±0.01	0.00	0.8±0.02	0.00	0.9±0.03
M. luteus	0.00	0.3±0.02	0.5±0.01	0.7±0.02	0.00	0.7±0.01	0.00	0.6±0.01
P. aeruginosa	0.2±0.03	0.3±0.02	0.00	0.4±0.03	0.00	0.5±0.02	0.00	0.6±0.01
P. multocida	0.7±0.06	0.6±0.04	0.00	0.9±0.02	0.00	0.7±0.01	0.00	0.7±0.03
P. vulgaris	0.1±0.03	0.3±0.02	0.00	0.3±0.02	0.00	0.3±0.02	0.00	0.5±0.02
S. aureus	0.00	0.3±0.02	0.6±0.05	1.6±0.04	0.4±0.06	1.9±0.03	0.6±0.05	1.6±0.02
S. epidermidis	0.2±0.03	0.5±0.01	0.4±0.05	0.9±0.02	0.1±0.03	1.4±0.01	0.00	1.3±0.01
S. typhi	0.3±0.05	1.0±0.01	0.00	1.0±0.01	0.00	1.0±0.02	0.00	0.7±0.03

(Clear zone in cm excluding 6 mm disc).

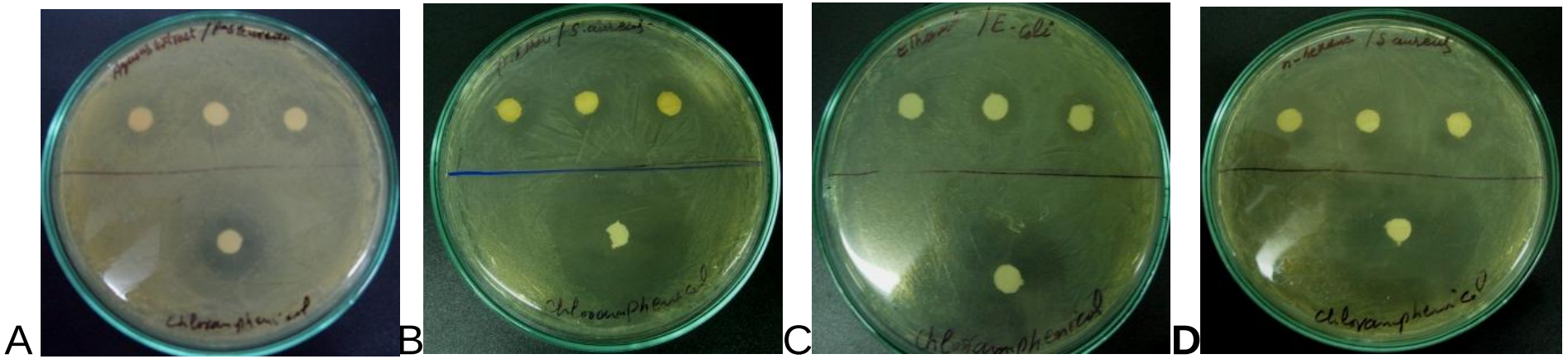

Plate 1. Antimicrobial activity of A:Aqueous, B:Petroleum ether, C:Ethanol and D:n-Hexane seed extract of *Momordica charantia L.* against *P. multocida, S. aureus, E. coli,S. aureus* respectively using zone inhibition assay.

G-25 separated fraction of the white seeds of *Cajanus cajan* were tested against pathogenic bacteria.

The antimicrobial activity amongst different isolated seed extracts, based on their total protein contents, was also checked against various pathogenic bacteria. The protein concentrations while applied on per disc (Tables 1 and 2) were not constant amongst various extracts; crude extract 625 µg/ml (19 µg / 30 µl) of, dialyzed ammonium sulphate pellet 683 µg/ml (21 µg / 30 µl), dialyzed ammonium sulphate supernatant

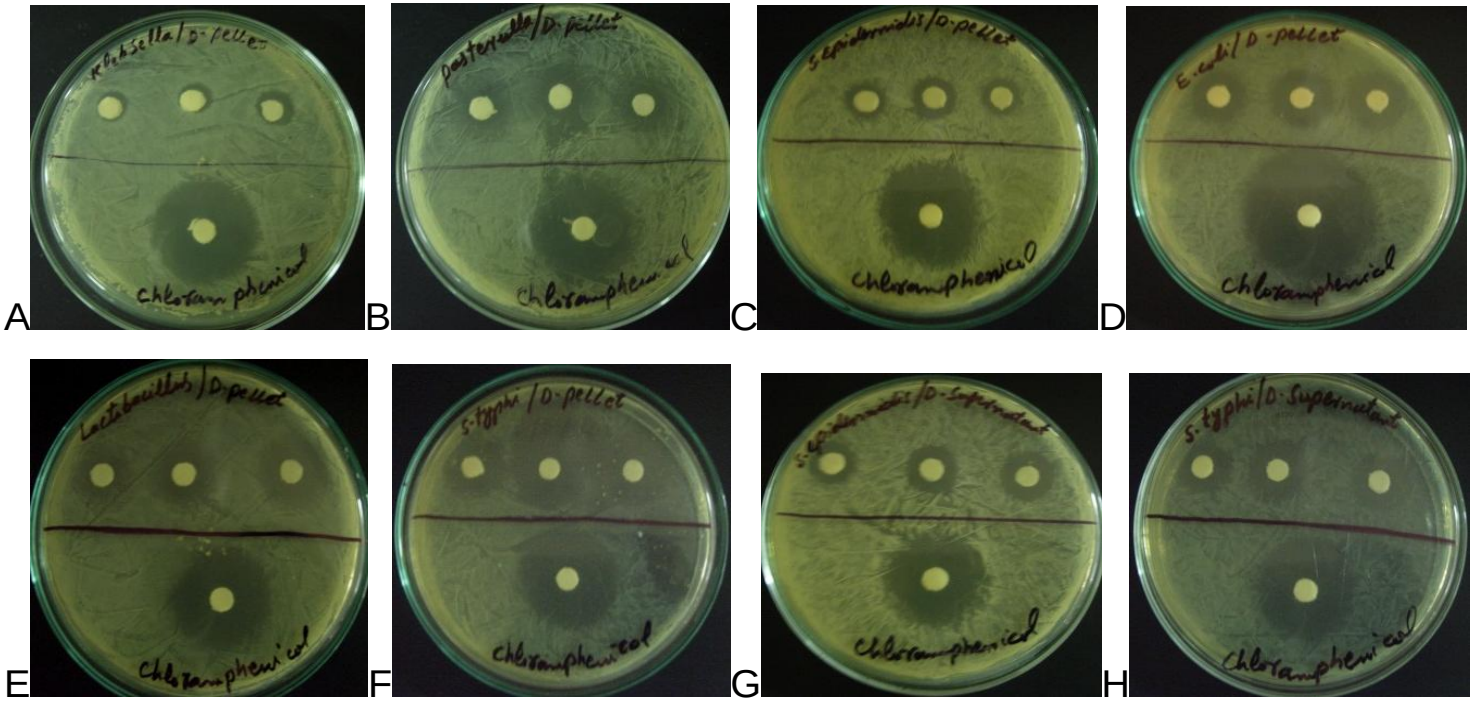

Plate 2. Antimicrobial activity of ammonium sulphate dialyzed pellet (A-F), supernatant (G,H) of seed extract of *Momordica charantia L.* against A: *K.pneumonae*, B*: P. multocida,* C*: S. epidermidis*, D: *E. coli,* E:*L. bulgaricus,* F: *S. typhi* using zone inhibition assay.

Table 2. Antimicrobial activity of the dialyzed pellet, supernatant and chromatographic fraction p22 of *Momordica charantia L.* seed extracts against pathogenic bacteria.

Bacteria	Dialyzed Ammonium Sulphate Pellet (683 µg /ml) (cm)	Control (cm)	Dialyzed Ammonium Sulphate Supernatant (379 µg /ml) (cm)	Control cm	Chromatography fraction p22 (290 µg /ml) (cm)	Control cm
E. coli	0.6.±0.05	1.5 ±0.03	0.6 ± 0.05	1.5 ±0.02	0.8 ± 0.02	2.0 ±0.01
K. pneumoniae	0.2±0.03	1.1 ±0.02	0.1 ± 0.02	0.9 ±0.03	0.8 ± 0.02	2.0 ±0.01
L. bulgaricus	0.6±0.05	1.0 ±0.01	0.2 ± 0.03	1.1 ±0.01	0.1 ± 0.01	1.0 ±0.02
M. luteus	0.1±0.01	0.8 ±0.01	0.00	0.6 ±0.03	1.0 ± 0.09	1.5 ±0.03
P. aeruginosa	0.00	0.6 ±0.02	0.00	0.5 ±0.03	0.00	1.0 ±0.01
P. multocida	0.3±0.06	1.1 ±0.01	0.1 ± 0.02	1.1 ±0.01	0.00	1.0 ±0.01
P. vulgaris	0.00	0.4 ±0.03	0.00	0.6 ±0.02	0.00	1.0 ±0.01
S. aureus	0.7±0.03	1.2 ±0.01	0.7 ± 0.03	1.3 ±0.02	1.0 ± 0.09	1.8 ±0.03
S. epidermidis	0.3±0.05	1.1 ±0.02	0.4± 0.05	1.0 ±0.01	0.1± 0.03	1.0 ±0.01
S. typhi	0.8±0.02	1.1 ±0.01	0.5 ± 0.04	1.0 ±0.01	1.0 ± 0.02	2.0 ±0.03

(Clear zone in cm excluding 6 mm disc).

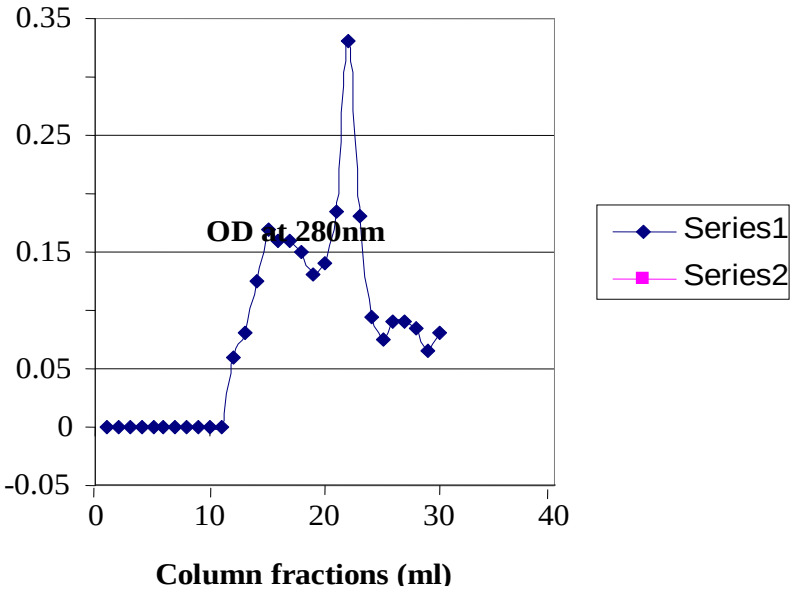

Figure 1. Gel filtration chromatography based protein profile of the dialyzed ammonium sulphate seed extract of *Momordica charantia L.*

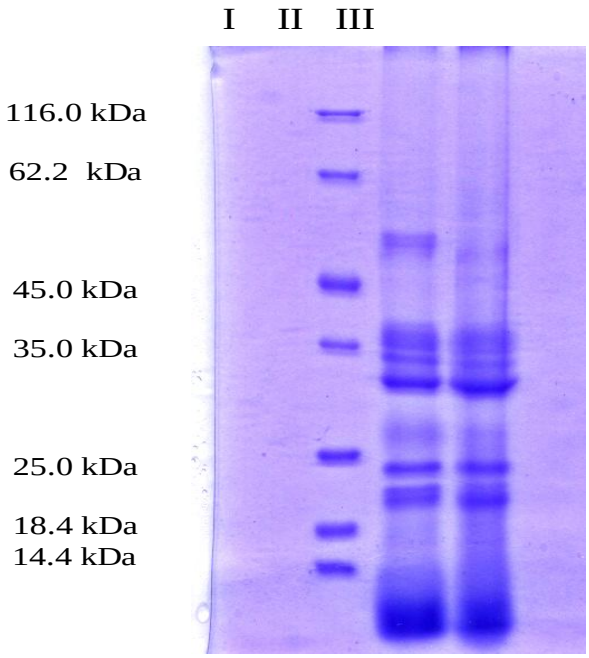

Figure 2. The molecular weight profile of aqueous extract and dialyzed ammonium sulphate pellet of *Momordica charantia*seed (II&III) on 12% SDS-PAGE along with molecular weight marker # 0431 in Lane I with a range of 14.4 – 116.0 kDa MW.

379 µg/ml (12 µg / 30 µl) and gel filtration column peak fraction 290 µg/ml (9 µg / 30 µl). Therefore, activity was estimated again by theoretically adjusting protein concentration in each sample as 25 µg / 30 µl. The adjusted protein concentrations based comparison resulted in highly significant antimicrobial activity with clear zone broader than control that is, chloramphenicol (Table 3).

Table 3. Antimicrobial activity at fixed protein concentration of dialyzed pellet, supernatant and chromatographic fraction p22 of *Momordica charantia L.* seed extracts against pathogenic bacteria.

Protein contents applied per disc (25 µg / 30 µl)	Aqueous seed Extract (cm)	Dialyzed ammonium sulphate pellet (cm)	Dialyzed ammonium Sulphate supernatant (cm)	Chromatography fraction p22 (cm)
E. coli	0.00	0.75	1.3	2.5
K. pneumoniae	0.13	0.25	0.23	2.5
L. bulgaricus	0.27	0.75	0.45	0.31
M. luteus	0.00	0.12	0.00	3.13
P. aeruginosa	0.27	0.00	0.00	0.00
P. multocida	1.0	0.38	0.23	0.00
P. vulgaris	0.13	0.00	0.00	0.00
S. aureus	0.00	0.88	1.59	3.13
S. epidermidis	0.27	0.38	0.90	0.31
S. typhi	0.41	1.0	1.14	6.3
Control	1.5	1.8	2.0	3.0

The protein concentration applied per disc was 25 µg/30 µl in each case (Clear zone in cm excluding 6 mm disc).

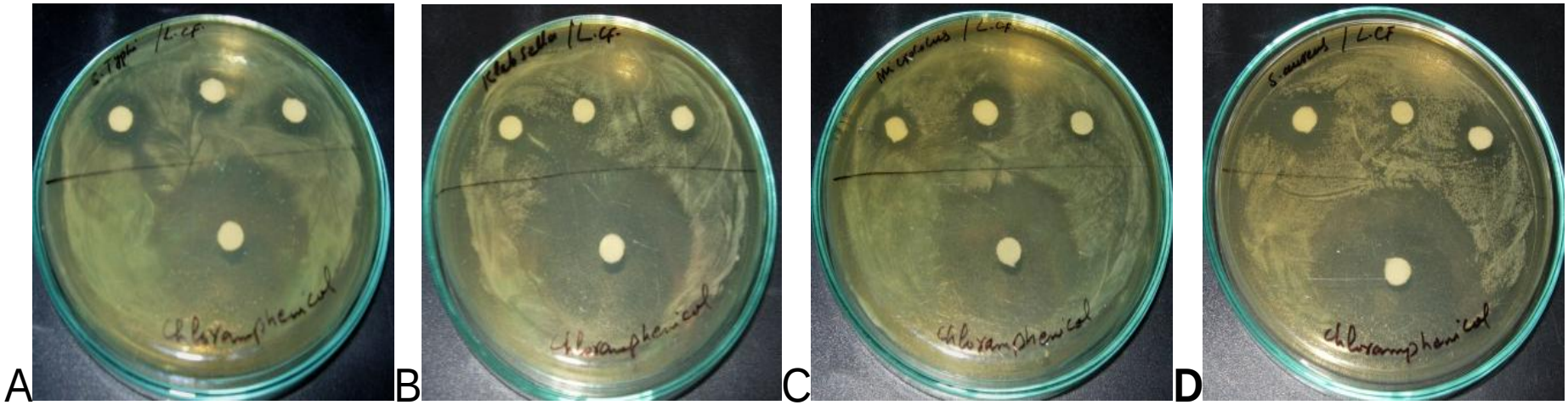

Plate 3. Antimicrobial activity of lypholized chromatographic fraction 22 extract of the seed of *Momordica charantia L.* against A: *S. typhi,* B: *K. pneumonae,* C: *M. luteus,* D: *S. aureus* using zone inhibition assay.

ACKNOWLEDGEMENT

We are thankful to Indigenous Scholarship Program, Higher Education Commission, Islamabad, Pakistan for providing funding to conduct this research work.

REFERENCES

Adekunle AA, Okoli SO (2002). Antifungal activity of the crude extract of *Alafia berteri* Oliver and *Chasmanthera dependens*. Hamdard Med., 55(3): 52-56.

Anwar Z, Ayub N, Khan AG (2000). Antibacterial ability of extracts from arbescular mycorrizal roots of *Allium sativum* L. and *Momordica charantia* L. Hamdard Med., 53(1): 29-33.

Dulger B, Gonuz A (2004). Antimicrobial activity of some Turkish plants. Pak. J. Biol. Sci., 7(19): 1559-1562.

Khan MR (1998). Broad spectrum antimicrobial activity of *Momordica charantia* and *Allium sativum*. Korean J. Pharmacogno., 29: 155-158.

Kight J (2003). Bitter Gourd or Melon - *M. charantia* guide to healthy herbs milot, brenda. herb clip: *Momordica charantia*. American Botanical Council Web-site. http://www.gerbalgram.org/herbclip/pdfs/050135-242.pdf. Accessed: September 10, 2005.

Ng TB, Chan WY, Yeung HW (1992). Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants. Gen. Pharmacol., 23(4): 579-590.

Onyeagba RA, Ugbogo OC, Okeke CU (2004). Studies on the antimicrobial effect of garlic, ginger and lime. Afr. J. Biotechnol., 3: 552-554.

Parasad G, Tyagi RK, Bhardwag DD, Gupta KK, Chopra AK (2002). Screening of antimicrobial potential of certain drug plants for enhancement of their therapeutic spectrum *in vitro*. Humdard Medicus, pp. 77-81.

Parkash A, Ng TB, Tso WW (2002). Purification and characterization of charantin, a napin like ribosome-inactivating peptide from bitter gourd (*M. charantia*) seeds. J. Pept. Res., 59(5): 197-202.

Rasheed A, Khan MR, Khalid N (1990). Antimicrobial activity of smaller proteins isolated from white seeds of *Cajanus cajan*. Pak. J. Biochem., pp. 23-25.

Sambrook J, Russell DW (2001). In: Molecular cloning- a laboratory manual, third edition (Cold spring Habour Laboratory) Cold Spring Harbour, N. Y. pp. A 8.40-8.51.

Sheng Q, Yao H, Xu H, Ling X, He T (2004). Isolation of plant insulin from *Momordica charantia* seeds by gel filtration and RP-HPLC. Braz. J. Med. Biol. Res., 27(6): 414-416.

Shinwari ZK, Malik S (1989). Plant wealth of Dera Bughti area, Progressive Farming, 9: 39-42.