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MOMORDICA CHARANTIA L. (*bitter gourd*) AS A CANDIDATE FOR THE CONTROL OF BACTERIAL AND FUNGAL GROWTH

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The plants use for medicinal purposes have been experienced by an extensive proportion of population of population for many centuries. The present study was designed to formulate cost effective dietary interventions to prevent and treat certain microbial diseases. The current experiment was conducted for evaluation of antifungal and antibacterial effects of bitter gourd (*Momordica charantia* L.), which is locally named as Karella. All parts of bitter gourd were used including seeds, pulp and skin. Its methanolic extracts were taken to check its antibacterial efficacy against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa, Escherichia coli* and methicillin resistance *Staphylococcus aureus* (MRSA) by paper disc diffusion test. Antifungal efficacy against selected species of *Penicillium and Aspergillus* was done by Agar Well-Diffusion Method. Broth Dilution method and Agar Well-Diffusion Method were used to determine the minimum inhibitory concentration (MIC). Zones of inhibition diameters were 11mm, 7mm, 11mm and 9mm for *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli,* respectively. There was no zone of inhibition against MRSA. The zones of inhibition for bitter gourd seed were 12mm, 8mm, 12mm and 11mm for *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi,* respectively. The MIC of bitter gourd pulp against *Aspergillus niger* and *Penicillium expansum* was 400mg/ml and 600mg/ml, respectively. The MIC of bitter gourd seed against *Aspergillus niger* and *Penicillium expansum* was 300mg/ml and 400mg/ml, respectively. Taking together, bitter ground was found a cost effective antibacterial and antifungal agent, it may be used an effective alternate remedial option.
Keywords: Cucurbits, plant extract, medicinal properties, phytochemicals, drug formulation, ethnopharmacy, antimicrobials.

INTRODUCTION

In the past, the therapeutic benefits are strongly correlated with the consumption of an appropriate food. Hippocrates said, "Let food be the medicine and medicine be the food". Contrarily, with the progress due urbanization, industrialization, and globalization "food as medicine" philosophy becomes oblivion. Modern lifestyle causes less concentration on healthy nutrition which has led in the emergence of several life threatening disorders.

The role of dietary components has been brought once again to front position in preventing different illnesses and boosting the healthy life style in past decades (Subratty *et al.*, 2005). Plant extracts have been tested for anti-inflammatroy, anticancer treatment (Kiran *et al*., 2018). Additionally, Nizami *et al.* (2018) reported the use of different peppers in different preparations for the treatment of highly invasive enteropathogens like Campylobacters and Helicobacters.

To determine the presence of profuse amount of physiologically active components in food has been attaining an enormous consciousness in current years. The excess of such active compounds in food known as phytochemicals has the potential of improving the health status (Boeing *et al.*, 2012). Now this fact has been globally approval that there is a definite relationship between "nutrition" and "health" that results in the development of term nutraceutical or functional food, also expressed in a variety of other lexes like "medifoods," "vitafoods", "medicinal foods," or "pharma-foods". Despite of all the development occurred in the field of medical sciences, in recent past incidence of infectious diseases increased significantly. Worldwide, infectious diseases have been considered as the leading cause of death (Ahmad *et al*., 2014), Antibiotics remains the first choice to treat the infectious diseases. On the other hand, emergence of antibiotic resistance made the treatment of infectious diseases very difficult (Zhang *et al*., 2017). The crisis of antibiotic resistance is alarming, as incidence of multiple drug resistant (MDR) pathogen is increasing throughout the world. Emergence of MDR pathogen is much faster as compared to the pace of new drug development. Due to this reason, researchers are looking for various alternatives to fight infectious diseases and antimicrobial resistance.

Plants are still recognized as a potential source of drug discovery and immunomodulatory treatment (Hassan *et al.*, 2017). It has been estimated that natural products are used as vital ingredients in the formulation of almost 50% of drugs. Various herbs have been found with the medicinal properties like purgatives, narcotics and sudorific. In plants, the presence of phytochemicals and biological constituents has incredible impact on the drug industry (Mahmood *et al.*, 2018). The therapeutic effects of plants are due to some chemical compounds which can provoke definite physiological actions in human physique. Many plant parts such as roots, leaves and fruits comprise bioactive substances like alkaloidal constituents, peptides, essential oils, and long chain unsaturated aldehydes, hence building them an abundant source of medicine. Likewise, it has been designated that these bioactive components are much potent against human pathogens such as bacteria, viruses, and fungi which might be a substantial effort in therapeutic strategies (Nizami *et al.*, 2018).

Momordica charantia L. or bitter gourd was selected for evaluation of its antimicrobial efficacy due to its higher consumption in usual remedies. This plant is extensively disseminated all over the tropics and used as conventional therapy for numerous illnesses including curing of wounds, infections, measles, fevers and hepatitis (Grover and Yadav, 2004; Subratty *et al.*, 2005). The whole *Momordica charantia* plant, comprising the stem, fruits, leaves and seeds hold remedial values and retain curative actions such as antidiabetic, antioxidant, anti-HIV, antiviral, anticancer activities (Grover and Yadav, 2004). Although several studies had been carried out on *Momordica charantia* L., most of them were focused on the treatment of diabetes whereas only little studies were exerted for its antimicrobial activity, especially on fruit extract (Mwambete, 2009).

Bitter gourd a vine of family Cucurbitaceae is also known as Karella, balsam-pear or bitter squashes, bitter melon. Bitter gourd has chemical constituents, which hold broad-spectrum antimicrobial action and defend the individuals from several digestive tract ailments. Numerous phytochemicals extracted from bitter gourd have been stated to have *in-vitro* antibacterial activity against *Klebsiella, Escherichia coli* (*E. coli*), *Staphylococcus, Pseudomonas, Bacillus subtilus, Streptococcus, Salmonella, Entamoeba histolytica* (Leelaprakash *et al*., 2011) and antifungal activity and antiviral activity against herpes, polio viruses, coxsackie virus B3, HIV and Epstein-Barr viruses (Palamthodi *et al*., 2014). It has been detected that bitter gourd has rich supply of proteins, lipids, water and carbohydrates in addition to existence of phytonutrients like minerals, dietary fibers, vitamins and anti-oxidants. Almost 228 various components were isolated from various fragments of bitter gourd (Singh *et al.*, 2011).

Research in the isolated drug discovery and herbal medicine need to be sustained, in view of the risk of new incipient diseases like bird flu, sever acute respiratory syndrome (SARS) and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). Plants are a principle source of natural goods such as phyto-chemicals and herbal medicine. Due to existence of some chemicals such as flavonoids, phenols, terpenes, anthroquinones, isoflavones and glucosinolates having antioxidant potential, bitter gourd supposed to be valuable for health and inhibits from several diseases (Drewnowski and Gomez 2000). Various synthetic drugs be indebted their potency and discovery by way of an imitator of assemblies from the natural products which sequestered from plants instead of created drugs by organic chemists.

From this perceptive of beneficial effects of bitter gourd on health, present project was planned to illustrate its antibacterial and antifungal effects. Modern and updated investigations on the plant biology reported that most of the bitter gourd plant may inhibit the activity of an enzyme called guanylate cyclase, which is associated with progression various infections.

MATERIALS AND METHODS

Procurement and processing of raw materials**:** Fruits of bitter gourd were obtained from Vegetable Research Section, Ayuub Agriculture Research Institute (AARI), Faisalabad. Further identification and authentication of specimens was done in the Department of Botany, University of Agriculture, Faisalabad. The fruits were subjected to washing, peeling and isolation of seeds, and cutting of pulp into small pieces. The material was dried at room temperature and grounded further to fine powder. After preparation of powder, it was packed separately in air-tight plastic jars for further analysis.

Preparation of aqueous methanolic extract of bitter gourd**:** The grounded material was dipped and shook in 70% methanol for 72hrs. Subsequently, filtration of material was done by using sterile cloth to separate the solvent. After filtration the remaining bulk was dipped again in 70 % methanol. The whole material was shaken for 72hrs followed by filtration for the separation of solvent. The whole process was repeated thrice. By using rotary evaporator, the evaporation of collected solvent having the bitter gourd extract was done. Then in vacuum oven further evaporation was done and dry material was quantified.

Preparation of McFarland turbidity standard**:** To standardize the bacterial suspension density for sensitivity test, a turbidity standard of $BaSO_4$ equivalent to 0.5 McFarland standards was used.

Antibacterial activity**:** The antibacterial activity of aqueous methanol extracts towards standard reference strains of *E. coli, P. aeruginosa Salmonella typhi, S. aureus* and MRSA were determined by disc diffusion test (Nostro *et al*., 2000). The experiments repetition was done thrice time and the mean values with standard deviation were obtained. The MIC of bitter gourd extract was determined, which exhibited antibacterial activity as evaluated by disc diffusion test. The MIC was calculated by using agar well diffusion method (Basu *et al*., 2005).

Antifungal activity**:** The antifungal activity of the bitter gourd extracts was evaluated against *Penicillium and Aspergillus* selected species by the agar well diffusion method (Kalidindi *et al*., 2015). The MIC for antifungal activity was evaluated by using the broth dilution method (Kalidindi *et al*., 2015).

RESULTS

***Antibacterial and antifungal activity*:** The DMSO (negative control) presented no inhibiting effect where as positive controls exhibited zone inhibition diameter of 20mm (Linezolid) just in case of *Staphylococcus aureus* and 16mm (amoxicillin) in case of *E. coli*, 13mm for *Pseudomonas aeruginosa* and 15mm for *Salmonella typhi* (Table 1). The results of the DMSO negative control test, exhibited no inhibiting effect. whereas, positive controls presented inhibition zone diameter of 17mm (Nystatin) in case of *Aspergillus niger* and 16mm for *Penicillium expansum* (Table 2).

***Estimation of minimum inhibitory concentration*:** The MIC value of Aqueous Methanol Extract of bitter gourd seed against *E. coli*, *Salmonella typhi*, *S. aureus*, *P. aeruginosa* and MRSA as evaluated by Agar Well Diffusion Method is listed in Table 3. Clear zones of inhibition of bacterial growth were observed, in 0.4, 0.2, 0.2 and 0.4 mg/ml of bitter gourd seed extract in DMSO. No zone of inhibition was observed against MRSA. The MIC of bitter gourd seed against *Aspergillus niger* and *Penicillium expansum* was 300mg/ml and 400mg/ml, respectively.

The MIC value of Methanol Extract of bitter gourd pulp against *E. coli*, *Salmonella typhi*, *S. aureus*, *P. aeruginosa* and MRSA as determined by Agar Well Diffusion Method is listed in Table 4 and Table 5. Clear inhibitory zones of bacterial growth were present in 0.4, 0.2, 0.2 and 0.4 mg/ml of bitter gourd pulp extract in DMSO. Again, in case of pulp no zone of inhibition was observed against MRSA. The MIC

Table 1. Disc diffusion inhibition zone diameters produced by different preparations of bitter gourd against selected bacteria as compared to Ciprofloxacin as positive control.

Bacteria	Bitter gourd seed	Bitter gourd pulp	Control (Ciprofloxacin)
Staphylococcus aureus	12mm	15mm	≤ 15mm
Escherichia coli	8mm	7mm	≤ 15mm
Pseudomonas aeruginosa	12mm	15mm	≤ 15mm
Salmonella typhi	11mm	9mm	≤ 15mm

Table 2. Disc diffusion inhibition zone diameters produced by different preparations of bitter gourd against selected bacteria as compared to Ciprofloxacin as positive control.

Fungi	Bitter gourd pulp	Bitter gourd seed	Control (Ciprofloxacin)
Aspergillus niger	15mm	14mm	≤ 15mm
Penicillium expansum	15mm	13mm	≤ 15mm

Table 3. Minimum inhibitory concentration by well diffusion method (Bitter gourd seed).

Concentration (mg/ml)	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*
0.05	No ZI	No ZI	No ZI	No ZI
0.1	No ZI	No ZI	No ZI	No ZI
0.2	12mm	No ZI	No ZI	8mm
0.4	15mm	8mm	10mm	14mm
0.6	16mm	9mm	11mm	16mm
1.0	17mm	10mm	12mm	18mm

*ZI = Zone of inhibition; mm = millimeter

Table 4. Minimum inhibitory concentration by well diffusion method (Bitter gourd pulp).

Concentration (mg/ml)	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*
0.05	No ZI	No ZI	No ZI	No ZI
0.1	No ZI	No ZI	No ZI	No ZI
0.2	11mm	No ZI	No ZI	7mm
0.4	14mm	7mm	9mm	12mm
0.6	15mm	8mm	11mm	15mm
1.0	17mm	9mm	11mm	17mm

*ZI = Zone of inhibition; mm = millimeter of bitter gourd pulp against *Aspergillus niger* and *Penicillium expansum* was 400mg/ml and 600mg/ml, respectively.

Table 5. Minimum inhibitory concentration by Broth dilution method (Bitter gourd seed and pulp).

Concentration (mg/ml)	*Aspergillus niger*		*Penicillium expansum*	
	Seed	**Pulp**	**Seed**	**Pulp**
50	+++	++++	++++	+++++
100	++	+++	+++	++++
200	+	++	++	+++
300	No turbidity	+	+	++
400	No turbidity	No turbidity	No turbidity	+
600	No turbidity	No turbidity	No turbidity	No turbidity
800	No turbidity	No turbidity	No turbidity	No turbidity
1000	No turbidity	No turbidity	No turbidity	No turbidity

DISCUSSION

Vegetables are earliest dietary crops; the first cultivation was done in 2100BC by humans (Shih *et al*., 2006). In Southeast Asia, mainly east India is the central place for the origin of these vegetables and their diversity (Kim *et al*., 2012) and measured as an important dietary source for humans (Suruchi and Khanna, 2011). Pakistan is blessed with various kinds of atmospheric conditions and land. Thus, in Pakistan, a huge amount of vegetables is cultivated all around the year (Athar and Bokhari, 2006).

Several modules like diet based programs have been proposed by scientific investigation to control life threatening illnesses such as obesity, diabetes, diseases and hypercholesterolemia (Duh and Yen, 1997). Among these approaches, a favorable implementation is to use the functional foods nutraceuticals that not only recover user health and wellness but also disease risk rate decreases with the minimum amount of cost. Accordingly, functional foods can be used as conventional foods as they are enhanced or supplemented products having rich nutrient supply (Goli *et al*., 2005).

In the current study, the antibacterial activity of *7* was evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, MRSA and *E. coli*, and antifungal activity against *A. niger* and *P. expansum*. The methanolic extracts of bitter gourd skin/pulp and seed were used. The results revealed that bitter gourd has resilient antimicrobial activity against these selected bacteria. The bitter gourd has strong antifungal activity against selected fungi. The methanolic extracts of bitter gourd skin/pulp was effective against selected strains of fungi.

The antimicrobial activity of bitter gourd against many types of bacteria and fungi is well documented. In addition to anti-inflammatory (Kobori *et al*., 2008; Lii *et al*., 2009) and antioxidant activities (Wu and Ng, 2008), the plant possess extraordinary inhibitory properties for various species of micro-organisms particularly bacteria and fungi (Abalaka *et al*., 2001). The results of the current study are showing resemblance with the Mwambete (2009) who investigated the bitter gourd fruit and leaf crude extracts for its antimicrobial activity against different strains of microorganisms that were purely isolated from different clinical cases and on the reference strains. Comparatively wider antimicrobial spectrum of activity was shown by the crude extracts of methanol than the extracts of ether. This antimicrobial spectrum of activity was displayed by the lower concentration as 0.075mg/µl.

In vitro studies indicated that extracts of seeds of bitter gourd and a molecule analogous to MAP30 possess wide-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. These extracts are not only helpful in inhibition of bacterial activity but also minimize infection and growth of several viruses, including HIV (Jiratchariyakul *et al*., 2001), In some studies, aqueous extract antimicrobial activity was milder as compare to antimicrobial activity of methanolic extract, which definitely specifies that methanolic extract comprises of higher amount of active antimicrobial agents such as alkaloids, glycosides, volatile oils, all of these are present in more plentiful quantity in bitter gourd (Grover and Yadav, 2004).

Current findings are also validated the results of Leelaprakakash *et al*. (2011) who found different parts of bitter gourd exhibited reduction in growth of bacteria and may be used to manufacture antibacterial medicines. They could be predominantly helpful in the treatment of skin infections such as acne, wounds and psoriasis (Roopashree *et al*., 2008; Leelaprakash *et al*., 2011). The oil extracted from the seeds of bitter gourd also has anti-bacterial potential (Braca *et al*., 2007). Different fractions of leaves and fruit of bitter gourd have been examined earlier showing antifungal action (Jagessar *et al*., 2008; Mwambete 2009; Santos *et al*., 2012). Antifungal ribosome inactivating proteins have also been extracted from bitter gourd seeds, which are effective against *Fusarium oxysporum* and *Pythium aphanidermatum* (Wang *et al*., 2004).

Results of the current study are also comparable to the results of Leelaprakash *et al*. (2011), they investigated aqueous and methanol extracts of bitter gourd for *in vitro* antimicrobial and antioxidant activity. They observed bitter gourd extract against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Bacillus subtilis* by well diffusion methods and disc diffusion. Methanol extract showed maximum activity.

***Conclusion*:** In the present study, the bitter gourd was subjected for the evaluation of possible efficacy against important pathogenic bacteria and fungi. It is concluded that *Momordica charantia* L. (bitter gourd) locally named Karella showed antimicrobial activity against selected bacterial and fungal pathogens which is statistically comparable to the antibiotics commonly used to treat the infections, so it can be used as an alternate to antibiotics to combat the infectious diseases and antimicrobial resistance.

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