ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

In Vitro Evaluation of Antimicrobial Activity of Fruit Extracts of *Momordica charantia* Linn. In Benzene

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ABSTRACT

Medicinal plants are being exploited for the treatment of various ailments since ancient times. Many plants act as the antimicrobial agents and are used for the treatment of various bacterial diseases and infections. In the present study, the antimicrobial activity of *Momordica charantia* fruits was assessed against four bacterial strains *viz. Streptococcus pyogenes*, *E.coli*, *Klebsiella pneumoniae* and *Lactobacillus acidophilus* by using the Disc Diffusion Assay. Extraction of various phytoconstituents of plant was carried out by using Benzene as a solvent. Among the four bacterial strains tested, the antibacterial activity of the *Momordica charantia* was observed to be highest for the *Streptococcus pyogenes* with a zone of inhibition of 9.0mm at a concentration of 100mg/ml. Also, the antimicrobial action of the plant extract was found to be concentration dependent. *Lactobacillus acidophilus* showed the least susceptibility against the antimicrobial properties of the plant. The MIC (Minimum Inhibitory Concentration) of the plant against all the bacterial strains was also determined. The MIC value of the plant extract of *Momordica charantia* against *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *E.coli* and *Lactobacillus acidophilus* was observed to be 1.56, 3.12,1.56, 6.25mg/ml respectively.

Key words – Antimicrobial activity, *Momordica charantia*, Benzene, Microorganisms, Minimum inhibitory concentration.

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

INTRODUCTION

Antibiotics (derived from the greek word Antiviotika) are the chemical substances that can inhibit the growth of other microorganisms. Nature itself produces antibiotics. Many microorganisms, including bacteria, produce them as a defence mechanism to fight off attack by other bacteria. Antibiotics can be classified in two broad categories namely bacteristatic and bactericidal. The bacteristatic are the one which inhibits the growth of bacteria while bactericidal antibiotics act by actually killing them. Antibiotic like Erythromycin, a macrolide antibiotic is a classic example of a bacteristatic antibiotic, while penicillin (which attack on cell wall) and Rifamycins (that impair the functioning of bacterial enzymes) are usually bactericidal (Lederberg, 1957, and Arioli *et al*., 1981). The extracts of various plant parts *viz.* Fruits, roots, leaves, stem are being used for treatment of various bacterial infections alongwith pipeline analysis of upcoming potential drugs. People prefer naturally derived drugs over synthetic ones due to their minimum side effects and cost effectiveness. Over 50% of all modern clinical drugs are of plant origin and thus these plant products play an essential role in drug development in the pharmaceutical sciences (Doshi *et al.,* 2011).

Bacteria can be divided into Gram- positive and Gram- negative bacteria on the basis of the color they turn during gram staining. Gram- positive bacteria have thick peptidoglycan layer and thus retain the stain and appears dark blue or violate in color. This is in contrast to the gram- negative bacteria which have a rigid cell wall and thus cannot hold the crystal violet stain. In the present study *Streptococcus pyogenes* and *Lactobacillus acidophilus* are used as Gram-positive bacterial strain while *Klebsiella pneumoniae* and *Escherichia coli* as gram-negative strains.

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

Momordica charantia (belongs to family Cucurbitaceae) is a popularly used vegetable in Indian subcontinent, Southeast Asia, Africa, China and south America, grown for food and medicinal purpose (Kumar *et al.*, 2010). *Momordica charantia* Linn. (Karela) is herbaceous, tendril bearing vine, attaining a height of about 5m. The fruits of *Momordica charantia* are characterized by a strong bitter taste, narrower in shape, pointed ends and surface covered with jagged triangular 'teeth' and ridges with green colouration. The plant has been claimed to work in treating of diabetes, ulcer, malaria, and cardiovascular diseases (Ghosh, 2014). However the exact properties of this vegetable, that makes it so beneficial for health is not known. The plant is known to contain bitter chemicals like charantin, vicine, terpenoids and glycosides alongwith peptidoglycan-p, a plant insulin, which could be responsible for the medicinal properties that the plant possess. Efforts have been done by various researchers to demonstrate the antibacterial properties of the *Momordica charantia* with the help of a range of experiments. Yasilada *et al.*, 1999, showed that the fruit extract of karela has some antibacterial activity against the bacteria *Helicobacter pylori* which is well known to cause the stomach ulcers. In another study, the water, ethanol and methanol leaf extracts of *Momordica* are found to be especially useful against the microbes *E.Coli, Staphylococcus, pseudomonas,* and *salmonella* among others (Sankaranarayanan and Jolly, 1993).

With the above background, an attempt to find out the answers of following questions has been made in the present study- 1). To provide the evidence to accept or reject the theory that fruits of *Momordica charantia* contains antibacterial activity. 2). In case of evidences in support, to determine whether the intensity of action is same or different for gram-positive and gram-negative bacteria. 3).To determine the MIC (minimum inhibitory concentration) against

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

four bacterial strains *viz. Streptococcus pyogenes, E.coli, Klebsiella pneumoniae* and *Lactobacillus acidophilus.*

Material and methods-

The fruits of *Momordica charantia* were collected from local area of Rohtak, Haryana, India, which is located between the geographical coordinates of 28.54ºN, 76.34ºE to 28.90ºN, 76.57ºE, during the period of December – February 2017-18. The maximum and minimum temperature recorded during this period was 17-22ºC and 6-13ºC respectively. The collected fruits were then air dried and converted into powdered form by using a laboratory blender. The 50g of this power was then loaded into the Soxhlet apparatus and the extraction was carried out by using Benzene as a solvent for about 48h. The plant extract was then filtered through Whatman filter paper no. 1 and evaporated by a using a rotary evaporated and freeze dryer. The dried extract was then stored at -20ºC in a refrigerator until use.

Bacterial strains-

The microorganisms used in present study were as follows: Two gram-positive bacteria (*Streptococcus pyogenes* and *Lactobacillus acidophilus)* and two gram-negative bacteria (*E.coli* and *Klebsiella pneumoniae)* .The bacterial strains were taken from the laboratory, botany department, MDU, Rohtak, Haryana (India) and were checked for their optical densities. The stock culture bacteria were revived by inoculating in broth media and grown at 37ºC for overnight.

Antimicrobial screening-

The antimicrobial activity of the benzene extract of *Momordica charantia* fruits was determined by using the disc diffusion assay (Bauer *et al.*, 1966). Nutrient Agar Himedia was prepared by dissolving 14g in 500ml of water. The media was then autoclaved and poured into pre-sterilized petri plates inside the

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

laminar air flow and kept for 15 min. The pre-sterilized discs, having diameter of 6mm were impregnated with 10µl of extract solutions of various concentration *viz.* 100, 50, 25 and 12.5mg/ml. These discs were then air-dried and placed on the surface of the agar plates. Ampicillin (10µg/disc) was used as a positive control while negative control was prepared by using the sterilized disc only. These plates were incubated at 37ºC for 24h, Thereafter, the zone of clearing were measured by using the antibacterial zone scale.

MIC (MINIMUM INHIBITORY CONCENTRATION) ASSAY-

The Minimum inhibitory concentration can be defined as the lowest concentration of the plant extract that can inhibit the visible growth of a microorganism after overnight incubation (Andrews JM, 2002). This can be determined by using the Micro broth dilution technique. An aliquot (100µl) of the extract was added to the 100 µl of nutrient broth. Subsequently, 50µl from the first well was transferred to the second well and this continued up to the seventh well. Thereafter, 10µl of the test organism and an equal amount of standardized resazurin is added into each well and mixed thoroughly. The MIC plate was then incubated at 37ºC for 24h (Akinpelu and Kolawole, 2004). The lowest concentration of the extract that results a change in the color is known as Minimum inhibitory concentration for that particular bacteria.

RESULT:

The antibacterial activity of benzene fruit extract of *Momordica charantia* was assayed against four bacterial strains by using the Agar disc diffusion method. The results obtained are tabulated in table 1.

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

Conc. Of plant extract (mg/ml)	Zone of inhibition(mm) Test Organism				Antibiotic activity
	Streptococcus pyogenes	*Klebsiella pneumoniae*	*E.coli*	Lactobacillus *acidophilus*	
12.5	6.5	6.5	7.0	6.0	Positive
25	7.0	6.5	7.0	6.5	Positive
50	7.5	6.5	7.5	6.5	Positive
100	9.0	8.0	8.5	7.0	Positive
Ampicillin	15.0	18.0	14.5	15.0	Positive

An analysis of these data shows that both, gram positive and gram negative bacteria are sensitive towards the plant extract. The highest antimicrobial activity was observed against the bacteria *Streptococcus pyogenes* (9.0mm), followed by *Escherichia coli* (8.5mm). According to Gislene *et al.*, *(*2000), any chemical that demonstrate the zone of inhibition of ≥7mm, is acceptable as being active. The antibacterial activity of the fruit extract is found to be concentration dependent as the zone of clearance increased with increasing concentration of the extract. This finding is in compliance with the studies carried out by Azu and Onyeogha (2007), which observed the concentration dependency of plant extract for being antimicrobial in action. Further, the plant extract is observed to be much less effective as compared to the reference

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

antibiotic Ampicillin. This may be due to the fact that insufficient quantities of active compound(s) are present in the crude extracts as compared to pure, standard antibiotics (Taylor *et al.*, 2001).

The result of MIC (Minimum Inhibitory Concentration) obtained when the benzene fruit extract of *Momordica charantia* was tested against four bacterial strains is shown in table 2.

Test organism	MIC(mg/ml)
Streptococcus pyogenes	1.56
Klebsiella pneumoniae	3.12
E.coli	1.56
Lactobacillus acidophilus	6.25

The highest MIC value obtained was for *Lactobacillus acidophilus* (6.25mg/ml), hence the bacteria is least sensitive towards the antimicrobial properties of the plant extract when compared to other bacterial strains. The plant extract is equally effective against *Streptococcus pyogenes* and *E.coli.*

Discussion:

The investigated fruit extract of *Momordica charantia* in benzene solvent did not show strong antibacterial activity. However there is low to moderate inhibitory action on microorganisms have been reported during the study. Literature data regarding the antimicrobial use of *M. Charantia* in Benzene solvent is quite limited, however, the Ethanolic and acetonic extract of this plant is shown to have considerably high antimicrobial activity in various studies carried out by researchers in all over the world. Adegbola *et al.*, 2016 observed the zone of inhibition of 28, 26, 24, 26, 26 and 24mm for *Staphylococcus*

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

aureus, E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi and *Klebsiella pneumoniae* respectively in ethanolic extract of fruits of *Momordica charantia.* Hence ethanolic extract shows a high and wide range of antimicrobial activity against all of the bacterial strains as compared to benzene fruit extract. This difference may be due to the extraction of different phytoconstituents in different solvents. It should be bear in mind that the antimicrobial activity of a plant is due to various bioconstituents like Glycosides, saponins, tannins, alkaloids and steroids. Since, the extraction of these phytoconstituents depends upon the type of solvent being used during extraction, so the finding of most appropriate solvent for effective action of medical plants needs to be keeps going. Such studies need to be carried out so that ignorance or acceptance of a particular solvent in view of its low or high potency in antimicrobial action of a specific plant can be done in further studies.

Conclusion:

From the present study it can be concluded that the investigated plant showed low to moderate antimicrobial activity against all the bacterial strains used. The results of current study are agreed to some extent with the traditionally observed medicinal use of *Momordica charantia.* The present results will form the basis for selection of solvents for particular plant species during future investigations.

Acknowledgement:

We are indebted to Prof. Pushpa Dahiya, Dean of life sciences and staff of the department of Botany, MDU, Rohtak, Haryana (India), for their constant help and support in conducting this work to full satisfaction. We also express gratitude to the university administration for providing the financial support for this course of work.

ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

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ISSN: 2278-4632
Vol-10 Issue-8 No. 5 August 2020

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