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Research paper

Phenolic Profile and Biological Properties of *Momordica charantia*

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Abstract: *M. charantia* is an important medicinal plant belongs to family *cucurbitaceae*. It originates from India, Malasiya and is widely spread all over tropical, subtropical and warm temperate regions of the world. This research work has been designed to evaluate the antioxidant, antimicrobial and toxicological potential of *M. charantia.* The antifungal and antioxidant components of *M. charantia* leaves, seeds and peels were extracted by using four solvent systems (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) andleaves presented maximum extract yield (22.7 g/100g DW) in 80% methanolic solvent system. Phytochemical analysis of *M. Charantia* leaves, seeds and peels extracts performed in terms of total phenolic and total flavonoid contents, showed that 80% methanolic leaves extract offered highest total phenolic contents (47.1 mg GAE/g DW), whereas80% ethanolic leaves gave maximum total flavonoid contents (67.3 mg CE/g DW). The phenolic contents were also analysed by HPLC. Antioxidant activity was determined by DPPH radical scavenging activity and measure of reducing power. Results revealed that 80% methanolic leaves extract showed highest radical scavenging activity and reducing potential. Antimicrobial activity of *M. charantia* leaves, seeds and peels was investigated by Disc Diffusion Method and Minimum Inhibitory Concentration (MIC). Results showed that 80% methanolic extract of leaves exhibited highest antibacterial and antifungal potential against *P. multocida* (30 mm DIZ) and *A. paraciticus* (28 mm DIZ), respectively. Cytotoxicity analysis was performed on BHK-21 cell by adopting the MTT assay. The cytotoxicity activity of the 80% methanolic extract of leaves was evaluated by noticing the cell survival percentage (53.4%). Overall results of the present study showed that 80% methanolic leaves extracts of *M. charantia* possesses very good antioxidant, antimicrobial and cytotoxic properties.

Keywords: *Momordica charantia*, General Description, Medicinal Properties

1. Introduction

Momordica charantia commonly known as bitter melon or bitter gourd is tropical or subtropical climber of the family of *cucurbitaceae* [1, 5]. It is mostly available in China, Malaysia, India and tropical Africa. All parts of the plant, including the fruit, taste is very bitter, as it contains a bitter compound called *momordicin* that is believed to have a stomachic effect [15, 19]. Bitter gourd has been used as folk medicine to cure toothache, diarrhea, furuncle, diabetes, dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism and scabies [3]. The Latin name *Momordica* means "to bite," referring to the jagged edges of the leaves, which appear as if they have been bitten [35].

The last few decades several hundred studies that have been carried with *M. charantia*. *M charantia* possess antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, anti-mycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic,

hypotensive, immunostimulant and insecticidal properties [18]. Traditionally, it is believed to have hypoglycemic effect. *M. charantia* containing food dishes are not popular in the Western world due to its taste [46, 47]. Although the different parts of plant *M. charantia* have been used as foodand drug but the fruit is the most important part. Depending onthe maturity stages, *M. charantia*seeds (BGS) have been found as a rich sourceof oil (18.1-37.6%) and protein (28-30%) [6, 69]. Previous investigations have shown that the fruits and leaves of *M. charantia* had rich phenolics and exhibited a high antioxidant activity. Fresh bitter melon is also used as a nourishing food, as it contains: 93.8% water, 0.9% protein, 0.1% lipid, 3.3% dietary fiber, 20 kJ energy per 100 g, 0.6% ash, and a small quantity, 0.05%, of vitamin C [70]. The nutritional value is less due to low levels of proteins and carbohydrates, as compared to other member of *Cucurbitaceae* with best nutritional value, might be due to the phosphorus, iron, and ascorbic acid content [17]. *M. charantia* plant is used as medicine and is a good nutritious source of phytochemicals [38]. The residue of alcoholic ether extract of *M. charantia* leaves, is reported to possess hypoglycemic activity. P-insulin (the pure protein) of *M. charantia* fruits plant part is also tested as in crystalline form [34].

Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones and glucosinolates [41]. Alteration in the total antioxidant content and activity due to different processing methods is of scientific importance as it has a direct impact on dietary nutrition. Blanching is a treatment of vegetables to inactivate enzymes such as polyphenol oxidase, catalase, peroxidase, lipogenase and chlorophylase [7, 8]. The antioxidant enzymes (AOE) include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and indirectly glutathione reductase (GR). Their role as protective enzymes are well known and have been extensively investigated both in vivo and in vitro in model systems [54]. Studies have reported that phenolics have potent antioxidant and free radical-scavenging activities. Whole bitter melon (flesh, aril and seeds) has been shown to be a good source of phenolic compounds and one study demonstrated that the flesh, aril and seeds all had very high antioxidant activity [64].

Many dietary phytochemicals, particularly phenolic compounds, have demonstrated antioxidant characteristics in various disease states such as diabetes, liver disease, cardiovascular disease and cancer. Consequently, the commercial development of plants as sources of antioxidants for health and nutritional purposes is of great interest worldwide [30]. Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates [41].

Colon rectal cancer is the second leading cause of cancer related deaths and the third most commonly occurring noncutaneous carcinoma in the United States of America [36]. *M. charantia* is rich in various biologically active chemicals including triterpenes, proteins, and steroids. Triterpenes of *M. charantia* has the ability to inhibit the enzyme guanylate cyclase that is thought to be linked to the cause of psoriasis [20]. In addition, guanylate cyclase is one of the important enzymes, necessary for the growth of leukemia and other cancer cells. In addition to these biologically active triterpenes, *M. charantia* proteins like momordin, alpha- and beta *momorcharin* and *cucurbitacin* B were also tested for possible anticancerous effects [26].

The anti-cancer properties of *M. charantia* are recently elucidated. Many researchers have found that treatment of *M. charantia*related products in a number of cancer cell lines induces cell cycle arrest and apoptosis without affecting normal cell growth [57]. The role of free radicals and active oxygen in treating chronic diseases including cancer, aging and atherosclerosis has been recognized [37]. Most recently, the same research group extended the antitumor potential of *M. charantia* crude extract to prostate cancer based on results from both in vitro and in vivo studies. It was observed that crude *M. charantia* extracts impaired cell cycle progression and inhibited xenograft proliferation [15]. The medicinal values of *M. charantia* have bioactive phytochemical constituents with non nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. In *M. charantia* primary metabolites are common sugars, proteins and chlorophyll while secondary metabolites are alkaloids, flavonoids, tannins and so on [10]. The aims of this study are to find the phenolic contents present in *M. charantia* its potentail antimicrobial and biological effects by using DPPH and MIC methods [11].

2. Material and Method

2.1. Chemicals and Standard Compounds

Butylatedhydroxytoluene (BHT) (99.0%), DPPH, catechin, gallic acid and Folin-Ciocalteu reagents were brought from Sigma Chemicals Co. (St, Louis, MO, USA). All standard antibiotic andculture media were bought from Oxoid Ltd., Immunoaffinity column (AflaTest® WB VICAM, USA) (Hampshire, UK).

2.1.1. Collection of Plant Materials

M. charantia leaves, seeds and peels were obtained from the vicinity of Lahore Garrison University, Lahore, Pakistan.

2.1.2. Preparation of Extract

For extraction of bioactive compounds, four solvent systems (100% methanol, 80% methanol, 100% ethanol and 80% ethanol) were being used. In this regard powdered seeds and leaves (20g) were extracted with 200mLin an orbital shaker for 8 hours at room temperature (Gallenkamp, UK). In order to separate the extract from residue whatman No. 1 filter paper was used. The resulting residues were extracted two times with the same solvent system. The drying of extracts was done at temperature of 45°C and their yield was calculated by weighing extracts. The extracts were kept in a refrigerator at 4°C for further analysis [20]. The ground samples were kept in polythene bags at 4°C until for further analysis.

2.1.3. Determination of Total Phenolic and Flavonoid Ciontents

By using Folin-Ciocalteu reagent total phenolic contents were determined [71]. The plant crude extract of 50 mg was mixed with 0.5 mL of Folin-Ciocalteu reagent and then 7.5 mL deionized water was added. The mixture was kept at room temperature for 10 min. Then 1.5 mL of 20% sodium carbonate (w/v) was added. At 40°C the mixture was in a water bath for 20 min and then cooled in an ice bath. Absorbance was measured at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Using gallic acid calibration curve the amounts of TP were calculated. The results were expressed as mg GAE/g DW. In thrice time all samples were analysed. The result was measured in average. All the results are reported on dried weight basis. Total flavonoid contents were determined by following the procedure of Dewanto *et al.* (2002). The aqueous extract of one milliliter of containing 0.01 g/mL of dry matter was placed in a 10 mL volumetric flask. Then 5 mL of distilled water was added followed by 0.3 mL of 5% $NaNO_2$. Then after 5 min, 0.6 mL of 10% $AlCl_3$ was added. After 5 min, 2 mL of 1 M NaOH was added. Then volume was made up with distilled water. After this the absorbance was measured at 510 nm. TF amounts were expressed as mg Catechin equivalent (CE)/g DW. All samples were analysed thrice and results were averaged [20, 24].

2.2. Analysis of Phenolics by High Performance Liquid Chromatography (HPLC)

2.2.1. HPLC Sample Extraction

With slight modifications Extraction/hydrolysis of phenolics was carried out following the method of [65]. Permanently acidified methanol (25 mL) containing 1% (v/v) HCl and 0.5 mg mL^{-1} TBHQ was added to each plant material (5 g). HCl (1.2 M, 5 mL) was added and the mixture was stirred at 90°C under reflux for 2 h. The extract was cooled to room temperature. Then centrifuged at 1500g (5000 rpm) for 10 min. Upper layer was taken and sonicated for 5 min, to remove air. Before injecting into HPLC, the final extract was filtered through a 0.45mL (Millipore) filter.

Using high-performance liquid chromatography the phenolic analysis was performed. An HPLC (model LC-10A, Shimadzu, Kyoto, Japan), CTO-10A column oven, equipped with two LC-10 AS pumps, Rheodyne injector, SCL-10A system control unit, SPD-10A UV–vis detector, and data acquisition class LC-10 software was used. A 20µL volume of the filtered sample was injected into an analytical Supelco (Supelco Inc., Supelco Park, Bellefonte, PA, USA) ODS reverse phase (C18) column (250×4.6 mm; 5µm particle size). Two solvent systems, a: contained water and Acetic acid (94:6 v/v) and B: contained 100% acetonitrile, were used. The chromatographic separation was performed by gradient elution of the mobile phase (0-15min= 15%B, 15-30=45%B, 30-45min=100%B) at a flow rate of 1.0 mL min^{-1} at room temperature. Detection was performed at a wavelength of 280nm. By comparing their retention times identification of phenolics compound was carried out with authentic standards (Sigma Chemicals Co., St Louis, MO, USA). Quantitative determination was carried out by using calibration curves of the standards [20].

2.2.2. DPPH Radical Scavenging Assay

By using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical then this wayfree radical scavenging activity was determined this described by [60]. This solution of DPPH (33mg/L) was prepared in methanol. This type of solution then this way the absorbance was taken at 0 min. Then thesolution of extract (250µg/mL) were prepared. Then 5mL of methanolic solution of DPPH was added in 1mL of extract solution. For 30 minutes the mixture was left in the dark. Then absorbance was measured at 517nm using a spectrophotometer. Then methanol used as blank. Free radical scavenging activity was expressed as percentage inhibition and calculated by using the following formula:

$$\text{Inhibition (\%)} = \text{Control Absorbance - Sample Absorbance} \times 100$$

Control Absorbance

Where control absorbance is absorbance of methanolic solution of DPPH taken at zero minute [60].

2.2.3. Reducing Power Determination

With slight modification then the reducing power of the plant leave extracts was determined according to the procedure described by [12]. Concentrated extract (2.5-10.0 mg) was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). Then the mixture was incubated at 50°C for 20 min. Then 5 mL of 10% trichloroacetic acid was added. Then the mixture was centrifuged at 980 *g* for 10 min at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 mL) was decanted and diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%). Then absorbance was checked at 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). All samples were analysed thrice and results averaged [41].

2.2.4. Evaluation of Antimicrobial Potential

The extracts of leaves and seeds were tested individually against a panel of microorganisms which may included five fungal strains (*Aspergillus parasiticus, Aspergillus flavus, Fusariumoryzae, Fusariumtritichum, Aspergillus oryzae*) and three bacterial strains (*Escherichia coli, Pasturellamultocida* and *Staphylococcus aureus*) obtained from the Fungal Bank, University of Punjab, Lahore. Fungal strains were cultured overnight at 28°C in Potato Dextrose agar (Oxoid Hampshire, UK), however the bacterial strain were cultured at 37°C in nutrient agar (Oxoid Hampshire, UK). The slants of microbial strains were stored at 4°C. Antimicrobial potential of plants extracts were determined by using the disc diffusion and microdilution broth assays [22, 25].

2.3. Disc Diffusion Assay

Antimicrobial activity of leaves, seeds and peels of *M.*

charantia was tested against fungal strains (*Aspergillus flavus, Aspergillus paraciticus, Fusariumoryzae, Fusariumtritichum, Aspergillus oryzae*) and bacterial strains (*Escherichia coli, Pasturellamultocida* and *Staphylococcus aureus*) by previously adopted method (NCCLS, 2004) with little modifications. The solution of Potato dextrose agar (PDA) was prepared and then autoclaved. Under laminar air flow, then about 20mL PDA solution was transferred in sterilized petri plate. Sterilized discs (6mm) of wicks sheet impregnated with 50µL of particular plant extract were placed on the agar plates. To equate the activity with standard antibiotics, as positive reference Fluconazol (30 µg/disc) (Oxoid) and Rifampicin (30 µg/disc) (Oxoid) were used as for fungal and bacterial strains respectively. As a negative control disc without samples were used. Standard disc and test discs and were placed in separate petri dishes. The plates were incubated at 28°C for 48h for fungal growth and 37°C for 24h for bacterial growth. Antimicrobial activity was evaluated by measuring the diameter of inhibition zones (mm) by zone reader [44].

2.4. Microdilution Broth Method

The method reported by [37] then this way MIC of plant extracts was evaluated. Briefly, in the first row 100µL of plant extract was transferred into 96 well microliter plates. To entirely other wells, 50µL of Sabarouraud dextrose broth and nutrient broth was added for fungal and bacterial strains, respectively [42]. Two-fold serial dilutions were performed using a micropipette such that each well had 50µL of the test material in serially descending concentrations. Finally, 10µL of microbial suspension was added to each well. Each plate had a row of negative control, a row of positive control of Fluconazole and Rifampicin for antifungal and antibacterial activities, respectively. The plates were prepared in triplicate and incubated at 28°C for 48h for fungi and 37°C for 24h for bacteria. By ELISA reader the absorbance was measured at 620 nm. At the lowest concentration then no growth was taken as the MIC value [51, 52, 53].

2.5. Toxicological Analysis

2.5.1. Cytotoxicity Assay

By adopting the MTT assay Cytotoxicity of plant extracts was evaluated then by using baby hamster kidney cells (BHK-21) as described by Freshney and Frame (1982), though 10% DMSO was used as a positive control. Solutions of the tested materials were evaluated for cytotoxic potential. The BHK-21 cells were revived using DMEM (Sigma-Aldrich, Germany) media as described by Freshney (1998). Then transferred into 40 ml cell culture flasks (Karrel Flasks, Corning, USA), which were then incubated for 72 h to get the confluent monolayer of cells. One hundred microliters of cell suspension (105 cells/ml) was dispensed into each well of 96-well plates (Corning, USA) and incubated at 37°C for 72 h. Media on the confluent monolayer of cells was regularly changed. Then 100 µl of the respective sample concentrations was added in triplicate, which was then incubated at 37°C for 48 h. Finally, the growth medium was removed. Then wells were washed with PBS and replenished with fresh media. One hundred microliters of 0.5% MTT solution was added to each well. Then each plates were incubated for ~4 h. The MTT solution was then removed, and plates were incubated at 37°C for 2 h. Thenthis after adding 5% DMSO to each well. Optical density was measured at 570 nm by an ELISA reader (Type355, Model 2005–05, Thermo, China) [6].

2.5.2. Statistical Analysis

By performing all experiments in triplicate (n=3), the data was described, mean ± SD. Data analyzed at 5% significant level by using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennsylvania, USA). As mean values at 95% confidence interval data of antifungal activity are presented. Significant differences of mean were calculated by using LSD [54, 55, 56].

3. Result and Discussion

3.1. Extracts Yields

The extraction yields from leaves and seeds and peels of using different solvent systems. Comparatively, 80% methanol exhibited higher extraction yields from leaves (22.7%). The extraction ability of different solvent systems for recovering extractable components from leaves followed the order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol. Yields of extract from seeds followed the order: absolute methanol >80% ethanol > 80% methanol>absolute ethanol. Yield of extract from peels followed the order: 80% methanol > 80% ethanol > absolute ethanol > absolute methanol [57].

3.2. Total phenolic and Total Flavonoid Contents

The total phenolic content from leaves, seeds and peels of using different solvent systems are presented in Table 1. Relatively, 80% methanol showed higher extraction yields from leaves (47.1%). The extraction capacity of different solvent systems for recovering extractable components from leaves followed order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol. Yields of extract from seeds followed the order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol. Yield of extract from peels followed the order: 80% methanol > 80% ethanol > absolute ethanol > absolute methanol [57, 58]. The total flavonoid content from leaves, seeds and peels of using different solvent systems are presented in Table 2. Moderately, 80% methanol exhibited higher extraction yields from leaves (67.3%). The extraction skill of different solvent systems for recovering extractable components from leaves followed the order: 80% ethanol > 80% methanol > absolute ethanol > absolute methanol. Yields of extract from seeds followed the order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol. Yields of extract from peels followed the order: 80% ethanol > 80% methanol > absolute ethanol > absolute methanol [59].

Table 1. *Total phenolic contents (mg GAE/g DW) of M. charantia seeds, peels and leaves extracts.*

Sr. no	Solvent System	Total phenolic contents (mg GAE/g DW)		
		Leaves	Seeds	Peels
1	80% Methanol	$47.1\pm0.56^{a}{}_{a}$	$36.2\pm0.42^{ab}{}_{b}$	$36.1\pm0.49^{a}{}_{b}$
2	80% Ethanol	$42.2\pm0.55^{ab}{}_{a}$	$35.3\pm0.46^{bc}{}_{b}$	$34.3\pm0.44^{b}{}_{c}$
3	100% Methanol	$40.3\pm0.50^{b}{}_{a}$	$34.5\pm0.59^{bc}{}_{b}$	$30.2\pm0.32^{d}{}_{c}$
4	100% Ethanol	$35.5\pm0.41^{bc}{}_{a}$	$27.1\pm0.45^{d}{}_{c}$	$32.1\pm0.45^{c}{}_{b}$

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different medicinal plants. Subscripts alphabets within the rows depicted significant difference among different plant parts.

Table 2. *Total flavonoid contents (mg CE/g DW) of M. charantiaseeds peels and leaves extracts.*

Sr. no	Solvent System	Total flavonoid contents (mg CE/g DW)		
		Leaves	Seeds	Peels
1	80% Methanol	$64.1\pm0.56^{ab}{}_{a}$	$57.4\pm0.42^{a}{}_{c}$	$61.2\pm0.49^{ab}{}_{b}$
2	80% Ethanol	$67.3\pm0.55^{a}{}_{a}$	$54.3\pm0.46^{b}{}_{c}$	$63.3\pm0.44^{a}{}_{b}$
3	100% Methanol	$57.2\pm0.50^{c}{}_{a}$	$49.2\pm0.59^{bc}{}_{c}$	$52.1\pm0.32^{d}{}_{b}$
4	100% Ethanol	$62.1\pm0.41^{bc}{}_{a}$	$45.1\pm0.45^{c}{}_{c}$	$57.4\pm0.45^{c}{}_{b}$

Values are mean ± SD of three solvents analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different solvents system. Subscripts alphabets within the rows depicted significant difference among different plant parts.

3.3. Phenolics Detected by HPLC

The HPLC examination of medicinal plant leaves revealed the presence of polyphenolic compounds like gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, *m*-coumaric acid, 4-hydroxy, 3-methoxy benzoic acid, ferulic acid, syringic acid and vanillic acid [50]. Phenolic compounds of *M. charantia* of different plant parts of leaves, seeds and peels are given in table 3. The total phenolic profile of *M. charantia*leaves revealed the presence of phenolic compounds like gallic acid (23.44ppm), chlorogenic acid (66.73 ppm) and *p*-coumaric acid is (53.21ppm). However *m*-coumaric acid (17.21ppm) and 4-hydroxy, 3-methoxy benzoic acid (403.7ppm) were found to be present in *M. charatia* peels. Moreover, syringic acid (56.34 ppm) and vanillic acid (301.9 ppm) were present in seeds of *M. charantia* [60, 61].

3.4. Antioxidant Activity

A large number of medicinal plants have beeninvestigated for their antioxidant properties. The natural antioxidants, either in the form of extracts or their chemical constituents are very important to prevent the destructive actions caused by oxidative stress [53].

3.5. DPPH Radical Scavenging Activity

Antioxidant property of the natural products is due to radical scavenging ability of phytoconstituents such as flavonoids, polyphenols, tannins, and phenolic terpenes [20]. Antioxident activity of these products can be evaluated by using DPPH radical scavenging assay which is widely used to test the ability of compounds that act as free radical scavengers or hydrogen donors [6]. DPPH is a stable nitrogen-centered free radical, its color changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are capable to perform this type of reaction can be considered as an antioxidants and therefore radical scavengers. It was also found that with increasing the DDPH radical scavenging activity of extract with concentration, antioxidant activity also increases [14]. So, DPPH (1, 1-diphenyl-2-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay which based on phenomenon of electron transfer that produces a violet colour in ethanol solution. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, give rise to colorless solution [15, 16]. Proton radical scavenging action is important mechanism for oxidation. The reduction capability of DPPH radicals was evaluated by the decrease in its absorbance at 517 nm, suggesting that antioxidant activity of plant extract is due to its proton donating ability [9, 10]. The hydrogen donating ability of the antioxidants molecules contributes to its free radical scavenging nature which is an important quality of antioxidants [52]. The DPPH radical assay has been used as a quick, reliable and easy method in order to investigate the general antioxidant activity of pure compounds as well as plant extracts [4]. It isa rapid method for screening of many samples for radical scavenging potential and is independent of sample polarity [13, 14].

Table 3. *Total Phenolic profile of M. charantia seeds, peels and leave.*

Plant Parts	Total Phenolics (ppm)			
	Gallic acid	Caffeic acid	4-hydroxy, 3-methoxy benzoic acid	p- coumaric acid
Leaves	23.44±0.04	ND	ND	53.21±0.05
Peels	ND	ND	403.7±0.04	ND
Seed	ND	ND	ND	ND

Table 3. *Contiuned.*

Plant Parts	Total Phenolics (ppm)					
	Ferulic acid	**m-coumaric acid**	**Chlorogenic acid**	**Syringic acid**	**Vanillic acid**	**Total Phenolics**
Leaves	ND	ND	66.73±0.01	ND	ND	166.54±0.04
Peels	ND	17.21±0.07	ND	ND	ND	420.91±0.05
Seed	ND	ND	ND	56.34±0.04	301.9±0.03	358.24±0.03

The DPPH radical scavenging activity from leaves, seeds and peels of using different solvent systems are presented in Table 4. Somewhat, 80% meth anolexhibited higher extraction yields from leaves (71.2%). The extraction ability of different solvent systems for recovering extractable components from leaves followed the order: 80% methanol > absolute ethanol > 80% ethanol > absolute methanol. Yields of extract from seeds followed the order: 80% ethanol >80% methanol> absolute ethanol > absolute methanol. Yields of extract from peels followed the order: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol [15, 16].

Table 4. *DPPH radical scavenging activity of M. Charantia seeds, peels and leaves extract.*

Sr. no	Solvent System	DPPH (%) radical scavenging activity		
		Leaves	**Seeds**	**Peels**
1	80% Methanol	$71.2\pm0.56^{a}{}_{a}$	$48.2\pm0.42^{c}{}_{c}$	$63.2\pm0.49^{a}{}_{b}$
2	80% Ethanol	$65.3\pm0.55^{bc}{}_{a}$	$51.3\pm0.46^{b}{}_{c}$	$60.3\pm0.44^{b}{}_{b}$
3	100% Methanol	$61.2\pm0.50^{c}{}_{a}$	$44.2\pm0.59^{e}{}_{c}$	$58.1\pm0.32^{bc}{}_{b}$
4	100% Ethanol	$67.5\pm0.41^{b}{}_{a}$	$46.1\pm0.45^{d}{}_{c}$	$53.2\pm0.45^{c}{}_{b}$

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different solvents. Subscripts alphabets within the rows depicted significant difference among different plant parts.

Reducing power

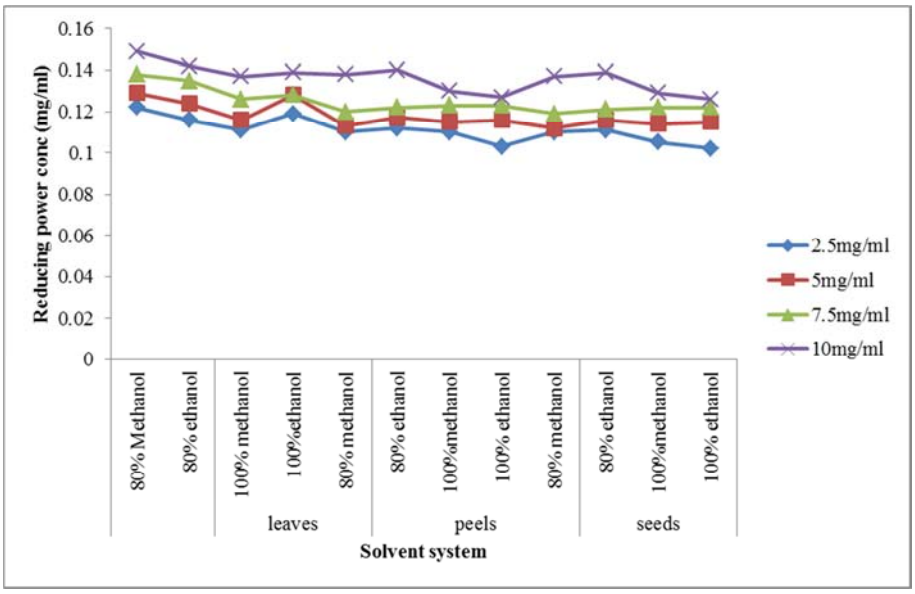

Figure 1. *Reducing power of M. charantiaseeds, peels and leaves extracts.*

Reductive abilities of the plant extracts can work as an indicator of their potential antioxidant activities [66, 67]. The antioxidant ability of phenolic compounds is generally due to their redox properties. Then this allows them to react as a reducing agent, an electron donor and an oxygen quencher. Study on medicinal plants and vegetables revealed that plant possesses the antioxidant activity and are capable of applying the protective effects against oxidative stress in biological systems [63, 64]. Antioxidants have the ability to donate electron to reactive radicals. Then they neutralize them into stable and nonreactive species [44, 45]. In this assay, the presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+} to the ferrous form. So reducing power can be measured by donating electron and reducing $Fe^{3+}(CN^{-})_6$ into $Fe^{2+}(CN^{-})_6$. The product of perl'sprussian blue color was formed that can monitor the Fe2+ concentration at the wavelength of 700 nm [2]. Then this was observed that higher absorbance value showed more reducing potential of medicinal plants [43, 44]. Hence, activity of reducing power increases with increasing the concentration of extracts [54, 55]. The result of the present investigation presented in Table 4 showed that leaves of *M. charantia* showed maximum reducing power [38].

3.6. Antimicrobial Activity

The antimicrobial activity of leaves and seeds extract of *M. charatia* was determined by disc diffusion and microdilution broth methods against a panel of micro-organisms [39].

3.7. Antibacterial Activity by Disc Diffusion Method

The antibacterial activity of leaves seeds and peels extracts of *M. Charantia* was determined by disc diffusion method against a panel of bacterial strains (*P. multocida, E. coli* and *S. aureus*). The antibacterial potential of leaves, seeds and peels extracts of *M. Charantia* varied significantly ($P< 0.05$) against bacterial strains as presented in table 5. The aqueous methanolic leaves extract was found to be the most effective against *P. Multocida* with DIZ of 30mm. However, absolute alcoholic leaves extracts exhibited no activity against *S. aureus*. The aqueous methanolic seeds extract showed highest DIZ (22mm) against *E. coli*. Whereas, all seed extracts showed no appreciable activity against *S. aureus*. Rifampicin was used as standard antibiotic. The Rifampicin showed DIZ (32mm, 29 mm and 20mm) against*P. multocida, E. coli* and *S. aureus* respectively [50, 51, 52].

Table 5. *Antibacterial activity of M. charantia seeds, peels and leaves extracts*

Plant parts	Microorganism	DIZ (mm)			
		80% Ethanol	Absolute ethanol	80% methanol	Absolute methanol
Leaves	*E. coli*	$25\pm1.30^{b}_{b}$	$20\pm1.66^{bc}_{d}$	$27\pm2.45^{bc}_{a}$	$23\pm2.0^{b}_{c}$
	P. multocida	$29\pm1.23^{a}_{b}$	$23\pm1.35^{b}_{d}$	$30\pm0.45^{a}_{a}$	$25\pm1.2^{a}_{c}$
	S. aureus	$13\pm1.78^{c}_{b}$	Nil	$14\pm1.51^{c}_{a}$	Nil
Seeds	*E. coli*	$22\pm0.67^{b}_{a}$	$17\pm0.98^{a}_{c}$	$22\pm1.63^{c}_{ab}$	$21\pm1.73^{a}_{b}$
	P. multocida	$21\pm0.55^{c}_{a}$	$16\pm1.25^{b}_{c}$	$17\pm1.95^{d}_{b}$	$15\pm1.22^{b}_{d}$
	S. aureus	Nil	Nil	Nil	Nil
Peels	*E. coli*	$21\pm0.67^{d}_{a}$	$15\pm0.98^{b}_{c}$	$20\pm1.63^{c}_{ab}$	$19\pm1.73^{a}_{b}$
	P. multocida	$22\pm0.55^{c}_{a}$	$18\pm1.25^{a}_{b}$	$15\pm1.95^{d}_{d}$	$18\pm1.22^{b}_{bc}$
	S. aureus	Nil	Nil	Nil	Nil

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05. Superscripts within the same column depicted significant difference among different fungal strains, while Subscripts within the same row indicated significant difference (p<0.05) between solvent systems.

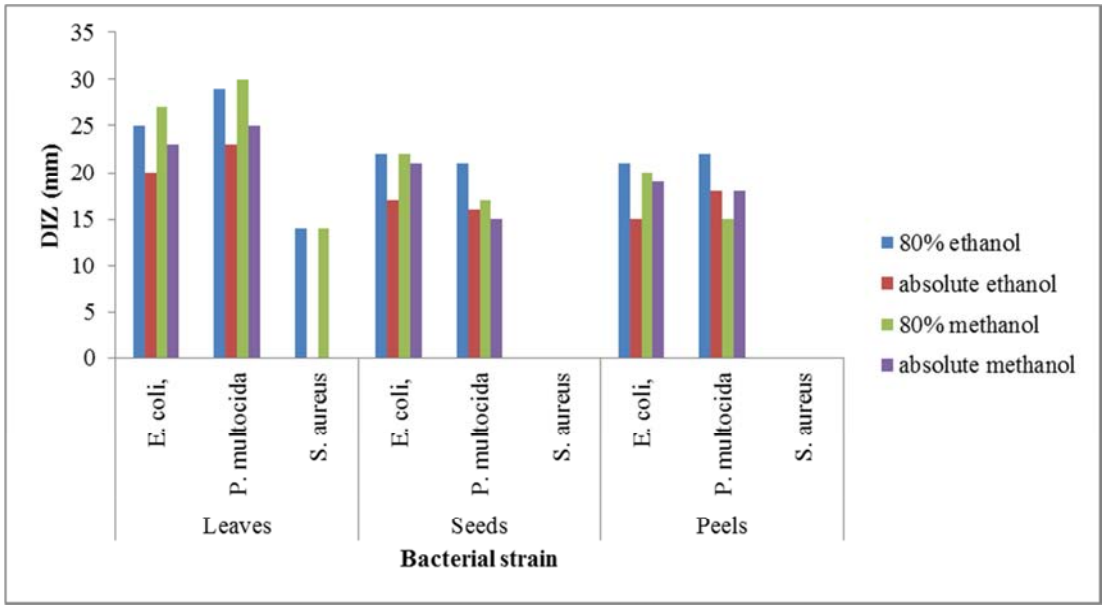

Figure 2. *Antibacterial activity of M. charantia seeds, peels and leaves extracts.*

3.8. Antifungal Activity Disc Diffusion Method

The antifungal activity of leaves, seeds and peels extracts of different parts of *M. Charatia* was determined by disc diffusion method against *A. parasiticus, A. flavus*, *F. oryzae, F. tritichum, A. Oryzae* [23-25]. The zones of inhibition are presented in table 6. It was concluded from the data that 80% ethanolic extract of leaves part showed strong activity against *A. Parasiticus* (28 mm zone of inhibition) nearly equal to control (27 mm) while 80% ethanolic leaves extract of peels part showed lowest activity against *A. oryzae* (9 mm zone of inhibition) [68]. It was observed that 80% ethanolic extracts of leaves are best antifungal source. Fluconazol was used as standard antibiotic [40]. The Fluconazol showed DIZ (31mm, 30 mm, 29mm, 28mm and 18mm) against *F. tritichum, A. parasiticus, A. flavus A. oryzae* and *F. oryzae* respectively [21, 26, 27].

Table 6. *Antifungal activity of M. charantia leaves, seeds and peels extract.*

Plant parts	Microorganism	DIZ (mm)			
		80% Ethanol	Absolute ethanol	80% methanol	Absolute methanol
	parasiticus	$28\pm1.13^{ab}{}_{a}$	$20\pm1.73^{c}{}_{c}$	$22\pm1.40^{bc}{}_{b}$	$16\pm2.07^{c}{}_{d}$
	flavus	$24\pm1.27^{b}{}_{a}$	$21\pm1.64^{c}{}_{c}$	$17\pm2.02^{cd}{}_{d}$	$22\pm1.85^{ab}{}_{b}$
Leaves	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$12\pm2.04^{cd}{}_{d}$	$13\pm1.81^{ef}{}_{c}$	$24\pm2.08^{ab}{}_{a}$	$18\pm2.09^{bc}{}_{b}$
	oryzae	$19\pm1.06^{cd}{}_{ab}$	$13\pm1.64^{ef}{}_{bc}$	$12\pm1.50^{f}{}_{cd}$	$12\pm2.05^{d}{}_{cd}$
	A. parasiticus	$20\pm1.31^{bc}{}_{b}$	$17\pm0.98^{ab}{}_{c}$	$22\pm1.63^{de}{}_{a}$	$15\pm2.02^{d}{}_{d}$
	flavus	$18\pm1.02^{ce}{}_{bc}$	$16\pm1.25^{cd}{}_{de}$	$12\pm1.95^{h}{}_{f}$	$17\pm2.08^{c}{}_{cd}$
Seeds	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$15\pm1.39^{ce}{}_{c}$	$15\pm2.07^{f}{}_{c}$	$17\pm1.55^{g}{}_{b}$	$24\pm1.69^{b}{}_{a}$
	oryzae	$10\pm1.95^{f}{}_{d}$	$16\pm1.54^{cd}{}_{b}$	$18\pm2.03^{ef}{}_{a}$	$12\pm1.89^{de}{}_{c}$
	parasiticus	$21\pm1.33^{ab}{}_{bc}$	$16\pm0.95^{b}{}_{f}$	$20\pm1.66^{bc}{}_{cd}$	$19^{cd}\pm2.07^{cd}{}_{de}$
	flavus	$20\pm1.01^{bc}{}_{a}$	$14\pm1.20^{bc}{}_{c}$	$13\pm1.94^{f}{}_{d}$	$18\pm2.09^{de}{}_{b}$
Peels	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$18\pm1.36^{cd}{}_{bb}$	$13\pm2.04^{e}{}_{d}$	$16\pm1.54^{e}{}_{cd}$	$29\pm1.67^{bc}{}_{ab}$
	oryzae	$9\pm1.93^{e}{}_{d}$	$17\pm1.59^{a}{}_{b}$	$19\pm2.04^{ce}{}_{a}$	$16\pm1.87^{ef}{}_{c}$

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05. Superscripts indicated significant difference (p<0.05) between fungal strains *while* subscripts alphabets within the row depicted significant difference among different solvent.

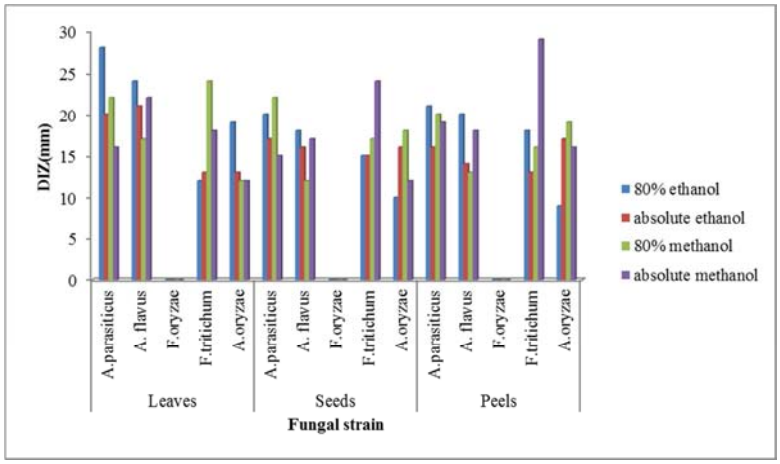

Figure 3. *Antifungal activity of M. Charantia leaves, seeds and peels extract.*

3.9. Minimum inhibitory Concentration (MIC) of M. Charantia Seeds, Peels and Leaves Extracts Against Bacterial Strains

MIC values of leaves, seeds and peels of *M. charantia* against bacterial strains are given in table 7. Data showed that the least MIC value was obtained from the absolute methanolic extract of seeds against *P. multocida*, showing its greater potential against *P. Multocida* [62]. While data showed that the least MIC value was obtained from the 80% ethanolic extract of leaves against *E. coli*, showing its lowest potential against *E. coli*. Seeds extracts were found to be ineffective against *S. aureus.* Rifampicin was used as standard antibiotic. The Rifampicin showed MIC (38µg/ml, 30µg/ml and 40µg/ml) against*P. Multocida, E. coli* and *S. aureus* respectively [28, 29, 30].

Table 7. *Minimum inhibitory concentration (MIC) of M. charantiaseeds, peels and leaves extracts ageist bacterial strains.*

Plant parts	Microorganism	MIC (µg/ml)			
		80% Ethanol	Absolute Ethanol	80% Methanol	Absolute Methanol
	E. coli	$34\pm1.04^{de}{}_{d}$	$45\pm1.08^{ef}{}_{c}$	$55\pm2.08^{ce}{}_{b}$	$67\pm2.09^{bc}{}_{a}$
Leaves	*P. multocida*	$40\pm0.87^{cd}{}_{cd}$	$53\pm1.26^{de}{}_{bc}$	$69\pm1.86^{bc}{}_{ab}$	$75\pm1.97^{ab}{}_{a}$
	S. aureus	$45\pm1.32^{bc}{}_{b}$	Nil	$73\pm1.55^{ab}{}_{a}$	Nil
	E. coli	$49\pm0.67^{b}{}_{d}$	$65\pm0.98^{bc}{}_{b}$	$62\pm1.63^{b}{}_{c}$	$71\pm1.77^{b}{}_{a}$
Seeds	*P. multocida*	$58\pm0.55^{a}{}_{d}$	$69\pm1.25^{a}{}_{bc}$	$73\pm1.95^{a}{}_{b}$	$85\pm2.05^{a}{}_{ab}$
	S. aureus	Nil	Nil	Nil	Nil
	E. coli	$42\pm0.67^{bc}{}_{d}$	$61\pm0.98^{ef}{}_{c}$	$66\pm1.63^{bc}{}_{b}$	$70\pm1.77^{cd}{}_{a}$
Peels	*P. multocida*	$54\pm0.55^{ab}{}_{d}$	$63\pm1.25^{cd}{}_{c}$	$76\pm1.95^{ab}{}_{b}$	$80\pm2.05^{bc}{}_{a}$
	S. aureus	Nil	Nil	Nil	Nil

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05. Superscripts indicated significant difference (p <0.05) between fungal strains *while* subscripts alphabets within the row depicted significant difference among different solvent.

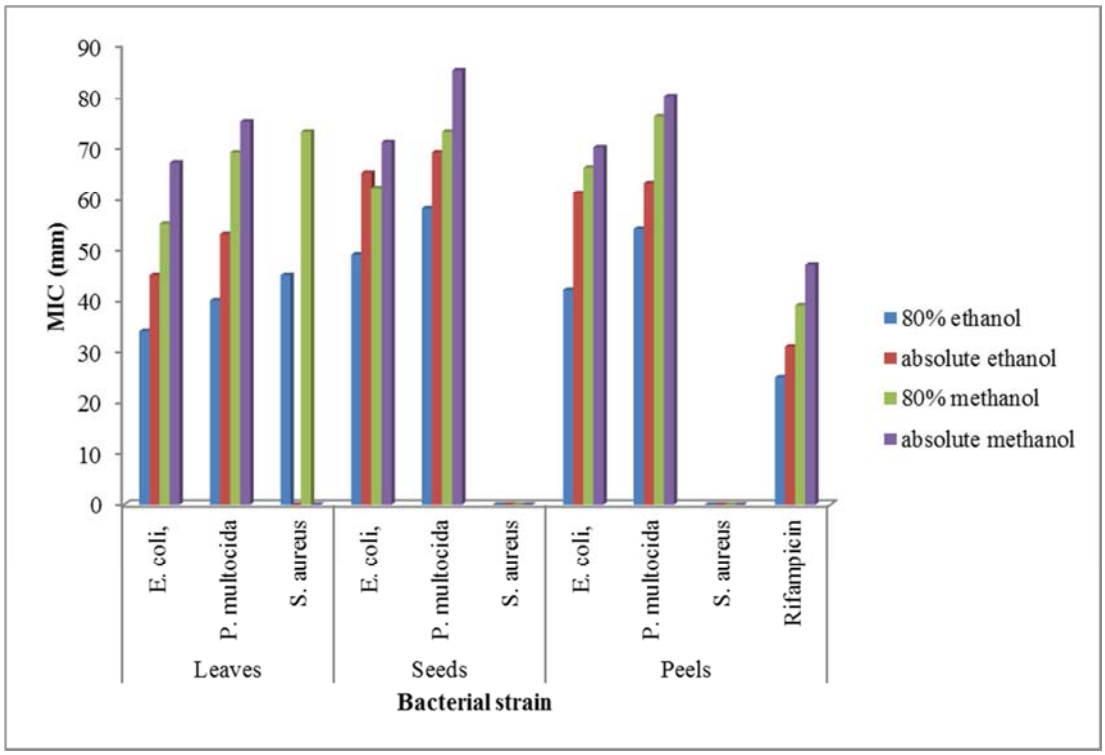

Figure 4. *Minimum inhibitory concentration (MIC) of M. charantiaseeds, peels and leaves extracts against bacterial strains.*

3.9.1. Minimum Inhibitory Concentration (MIC) of M. Charantia Seeds, Peels and Leaves Extractsagainst Fungal Strain

The minimum inhibitory concentration of the *M. charantia* extracts was determined against the test organisms. The minimum inhibitory concentrations are presented in the Table 8. From the data, it was concluded that 80% ethanolic extract of leaves part showed lowest minimum inhibitory concentration (34 µg/ml) against *A. Paraciticus* while the absolute methanolic extract of peels part showed highest concentration (106µg/ml) against *A. oryzae*. Fluconazol was used as standard antibiotic [48, 49]. The Fluconazol showed MIC (39 µg/ml, 30µg/ml, 32 µg/ml, 46µg/ml and 47µg/ml) against *F. tritichum, A. parasiticus, A. flavus A. oryzae* and *F. oryzae* respectively [31, 32, 33].

Table 8. *Minimum inhibitory concentration (MIC) of M. charantiaseeds, peels and leaves extracts against fungal strains.*

Plant parts	Microorganism	MIC (mm)			
		80% Ethanol	Absolute ethanol	80% methanol	Absolute methanol
	parasiticus	$34\pm1.13^{de}{}_{d}$	$36\pm2.73^{fg}{}_{c}$	$45\pm1.44^{ef}{}_{b}$	$47\pm2.08^{ef}{}_{a}$
	flavus	$38\pm1.27^{de}{}_{f}$	$42\pm2.67^{fg}{}_{cd}$	$48\pm2.05^{ef}{}_{bc}$	$52\pm1.85^{ce}{}_{ab}$
Leaves	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$46\pm2.07^{cd}{}_{f}$	$53\pm2.89^{ef}{}_{e}$	$67\pm2.45^{de}{}_{d}$	$78\pm2.09^{bc}{}_{c}$
	oryzae	$53\pm1.67^{cd}{}_{d}$	$57\pm2.24^{cd}{}_{c}$	$72\pm2.47^{cd}{}_{b}$	$82\pm1.93^{bc}{}_{a}$
	parasiticus	$55\pm1.33^{e}{}_{d}$	$64\pm2.07^{cd}{}_{c}$	$77\pm2.41^{cd}{}_{bc}$	$88\pm2.00^{bc}{}_{ab}$
	flavus	$62\pm1.09^{bc}{}_{d}$	$67\pm2.54^{c}{}_{cd}$	$79\pm2.59^{cd}{}_{b}$	$91\pm2.09^{bc}{}_{a}$
Seeds	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$74\pm1.41^{a}{}_{e}$	$75\pm2.09^{bc}{}_{d}$	$89\pm1.53^{bc}{}_{cd}$	$99\pm1.67^{ab}{}_{bc}$
	oryzae	$68\pm1.94^{b}{}_{d}$	$79\pm1.77^{ab}{}_{c}$	$92\pm2.04^{b}{}_{bb}$	$105\pm1.87^{a}{}_{ab}$
	parasiticus	$57\pm1.34^{cd}{}_{d}$	$65\pm2.08^{cd}{}_{c}$	$78\pm2.45^{e}{}_{b}$	$89\pm2.01^{cd}{}_{a}$
	flavus	$63\pm1.09^{bc}{}_{d}$	$68\pm2.55^{bc}{}_{cc}$	$81\pm2.58^{cd}{}_{bc}$	$92\pm2.09^{bc}{}_{ab}$
Peels	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	$75\pm1.42^{a}{}_{d}$	$76\pm2.09^{ab}{}_{c}$	$90\pm1.55^{bc}{}_{b}$	$98\pm1.69^{ab}{}_{a}$
	oryzae	$69\pm1.95^{ab}{}_{d}$	$80\pm1.78^{a}{}_{c}$	$93\pm2.06^{ab}{}_{bc}$	$106\pm1.89^{a}{}_{ab}$

Values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$. Superscripts indicated significant difference ($p < 0.05$) between fungal strains *while* subscripts alphabets within the row depicted significant differences among different solvent.

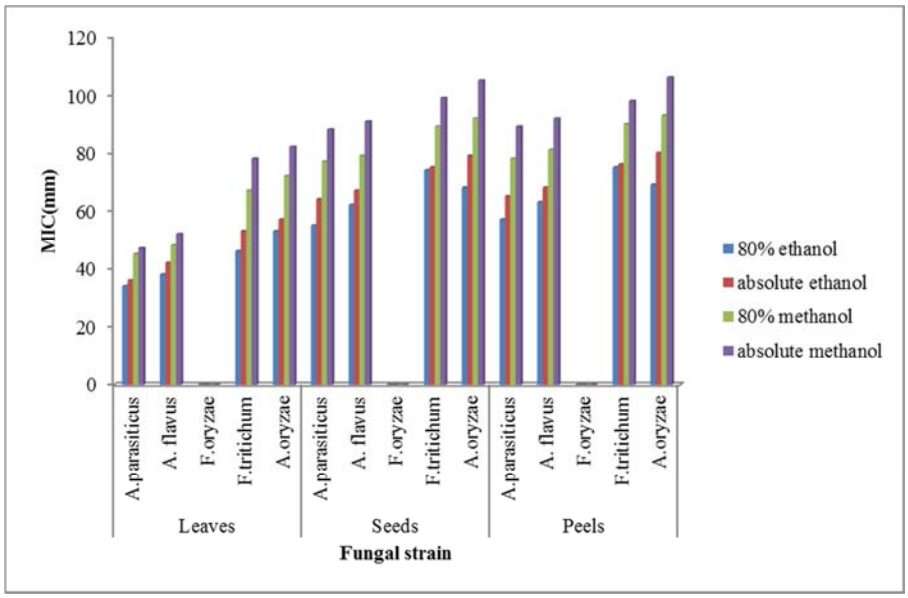

Figure 5. *Minimum inhibitory concentration (MIC) of M. charantia seeds, peels and leaves extracts against fungal strains.*

3.9.2. Cytotoxicity Assay

By adopting the MTT assay Cytotoxicity of plant extracts was evaluated then by using baby hamster kidney cells (BHK-21) as described by Freshney and Frame (1982), though 10% DMSO was used as a positive control. Solutions of the tested materials were evaluated for cytotoxic potential.

Table 9. *Cytotoxic potential of M. Charantia leaves.*

Medicinal plant extract	Treatment (g/ml)	Cell survivalPercentage (%)
80%Methanolic leavesextract	24710	53.40±0.27

4. Conclusions

In this study, it was confirmed that different parts of *M. charantia* species exhibited excellent biological potential. The phytochemical analysis was carried out and showed considerable amount of TFC and TPC in different plant parts of *M. charantia*. Moreoverleaves plant part exhibited effectiveantimicrobial and antioxidantactivity. The major pharmacological components present in the leaves extracts of different parts of *M. charantia* showed higher antimicrobial effects as well as antioxidant potential. In this way this might accelerate the development of new drugs for numerous diseases caused by microorganisms. The present research work is economic, cost effective and can be applied in food and pharmaceutical industries by forming antimicrobial and antioxidative reagents.

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