Comparative Study of Hydroalcoholic Extracts of *Momordica charantia* L. against Foodborne Pathogens

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The antimicrobial effect of 24 different hydroalcoholic extracts (100, 75, 50 and 25% methanol and water) obtained from four parts (leaf+stem (aerial), peel, pulp and seed) of *Momordica charantia* L. were investigated against five Gram-positive, six Gram-negative and four fungal strains. The extraction was done by individual cold percolation method using hexane, different hydroalcoholic solvent (100, 75, 50 and 25% methanol) and water. The antimicrobial activity was done by agar well diffusion assay. The extracts, which showed >15 mm zone of inhibition, were further screened to determine minimum inhibitory concentration and minimum bactericidal concentration using a broth dilution method performed in 96-well microtitre plate. The extractive yield was highest in aqueous extracts of all the four parts closely followed by 25% methanol. *Micrococcus flavus* was the most susceptible Gram-positive bacteria and *Pseudomonas testosteroni* was the most susceptible Gram-negative bacteria. The highest antibacterial activity was shown by 100% methanol. The Gram-negative *Pseudomonas* spp. was more susceptible towards all the extracts than the Gram-positive bacteria or fungal strains investigated. One hundred percent and 50% methanol extracts of seed showed lowest minimum inhibitory concentration and minimum bactericidal concentration values, that is <39 and 625 μg/ml, respectively, against *Pseudomonas pictorum*. Therefore, these extracts would be of interest in the control of *Pseudomonas* spp. in food industry as well as used for therapeutic purposes.

Key words: *Momordica charantia,* MIC, MBC, food spoilage, antipseudomonal activity, peel, hydroalcoholic extract

Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. Consumers today are increasingly concerned about chemical preservatives in food and tend to choose food products that are natural, safe and with multi-health benefits[1-3]. Foodborne illness is a major problem associated with enormous costs. Foodborne pathogens occur widely in nature and it is difficult to prevent them from entering raw foods. *Salmonella* sp., *Listeria monocytogenes, Bacillus subtilis* and *Escherichia coli* account for the largest number of outbreaks, cases and deaths, and are capable of attaching to inert surfaces and subsequently forming bio films on food processing equipment and environment[4,5]. *Staphylococcus aureus* causes a range of illnesses and was found to be the most resistant organism[6]. *Salmonella* mutants survive and are able to persist in the food chain[7]. Many *Pseudomonas* spp. can cause food spoilage. Novel antipseudomonal activity is of particular interest as it is the leading cause of nosocomial infections and has developed mechanisms of resistance to common classes of antibiotics[8,9]. The resistance of bacteria and other microorganisms to antimicrobial agents has become a wide-spread medical problem especially as nosocomial pathogens. To reduce health hazards and economic losses due to foodborne microorganisms, the use of natural products as antibacterial compounds is gaining importance. However, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines. Several plants have been reported to be used in treating and managing the complicated diseases.

The food antimicrobials are classified into natural and synthetic substances depending on their origin. Although, many synthetic antimicrobials are found naturally (benzoic acid in cranberries, sorbic acid in rowanberries, citric acid in lemons, malic acid in apples and tartaric acid in grapes), the perception of natural has become important for many consumers[10]. The problems mentioned introduced new research directions in the field of bioactive principles from

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natural sources and their application as food additives or dietary supplements.

Momordica charantia L. (Cucurbitaceae) commonly known as 'bitter gourd' and 'bitter melon', '*karela*' is a multipurpose herb widely cultivated in many tropical and subtropical regions of the world. The fruits are used as medicinal vegetable in different parts of the world. Apart from their role in food consumption, a wide array of pharmacological activities such as antidiabetic[11], antioxidant[12], anticancer activities[13] and antiulcer[14] are reported for this plant.

MATERIALS AND METHODS

Collection of the plant material:
Different parts (aerial, peel, pulp and seed) of *Momordica charantia* L. were collected in September 2011 from Chotila, Surendranagar, Gujarat, India and identified by comparison with specimens (PSN333) available at the Herbarium of the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The parts were separated, washed thoroughly with tap water, shade dried, homogenised to fine powder and stored in airtight bottle.

Hydroalcoholic extraction method:
The dried powders of all the four parts were extracted individually by cold percolation method[15-17]. The hydroalchoholic extraction was done using methanol and water[18]. The dried powder was first defatted by hexane and then extracted in 100% methanol (MeOH), 75% MeOH, 50% MeOH, 25% MeOH and 100% water (aqueous). Ten grams of dried powder was taken in 100 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 ml of each solvent, that is 100% MeOH, 75% MeOH, 50% MeOH, 25% MeOH and water in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min, the supernatant was collected and the solvents were partially evaporated using rotary vacuum evaporator (Equitron, India) then kept in petri plates to dry. The extract was stored at 4° in air tight bottles. The residues were weighed to obtain the extractive yield.

Antimicrobial activity:
The microorganisms used in this investigation were obtained from National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4°. The Gram-positive bacteria studied were *Staphylococcus aureus* ATCC29737 (*SA*), *Staphylococcus albus* NCIM 2178 (*SAL*), *Corynebacterium rubrum* ATCC14898 (*CR*), *Listeria monocytogenes* ATCC19112 (*LM*), *Micrococcus flavus* ATCC10240 (*MF*); Gram-negative bacteria used were *Pseudomonas aeruginosa* ATCC27853, (*PA*) *Pseudomonas stutzeri* NCIM5136 (*PSt*), *Pseudomonas pictorum* NCIB9152 (*PPi*), *Pseudomonas putida* NCIM2872 (*PP*), *Pseudomonas testosteroni* NCIM5098 (*PT*), *Pseudomonas syrigae* NCIM5102 (*PS*); and fungi were *Candida albicans* ATCC2091 (*CA*), *Candida neoformans* NCIM3542 (*CN*), *Candida glabrata* NCIM3448 (*CG*), *Candida epicola* NCIM3367 (*CE*). The organisms were maintained on nutrient agar and MGYP medium (Hi-Media, India) for bacteria and fungi respectively, at 4° and subcultured before use. The microorganisms studied are clinically important ones causing several infections and food spoilage. Ampicillin (AMP 10 µg/disc), chloramphenicol (CH 30 µg/disc), tetracycline (T 30 µg/disc), amphotericin B (AP 100 units/disc) and nystatin (NS 100 units/disc) were used as standard to determine antimicrobial susceptibility. Chloramphenicol and ceftazidime (CF) were used during minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination. All antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, India).

Agar well diffusion method:
In vitro antimicrobial activity of the different solvent extracts was studied against pathogenic microbial strains by the agar well diffusion method[19-22]. Mueller-Hinton No. 2/Sabouraud dextrose agar (Hi-Media) was used for the antibacterial and antifungal susceptibility test, respectively. The different solvent extracts were diluted in 100% dimethyl sulfoxide (DMSO) to give a concentration of 20 mg/ml. The Mueller-Hinton agar/Sabouraud dextrose agar was melted and cooled to 48–50° and a standardised inoculum (1.5×10^8 CFU/ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes;

wells (8.5 mm) were prepared in the seeded agar plates. The test compound (100 µl) was introduced into the well. The plates were incubated overnight at 37° and 28° for 24 and 48 h, respectively, for bacteria and fungi. DMSO was used as negative control. The microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented with ±SEM (standard error of mean).

Preparation of bacterial inocula and extracts or antibiotics for MIC and MBC study:

The inoculum of the test organisms were prepared using the colony suspension method[23]. Colonies picked from 24 h old cultures, grown on nutrient agar, were used to make suspension of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before use to yield 6×10^5 CFU/ml. Twofold serial dilutions using 100% DMSO were carried out from the 1250 µg/ml stock plant extract to make six test concentrations ranging from 39 to 1250 µg/ml for each solvent extracts. Twofold dilutions of chloramphenicol and ceftazidime (1–32 µg/ml) were used as a positive control.

Determination of minimum inhibitory concentration:

The MICs were determined only for the test organisms that had shown >15 mm zone of inhibition of the crude extracts. Micro broth dilution method performed in sterile flat bottom 96 well micro test plates (Tarsons Products Pvt. Ltd.) was performed to evaluate MIC of the plant extracts[24]. One hundred and fifty microlitres of Mueller-Hinton broth was introduced into all the 96 wells and 20 µl of varying concentrations of the extract was added in decreasing order along with 30 µl of the test organism suspension. A final volume of 200 µl was achieved in each well (150 µl Mueller-Hinton broth, 30 µl of the test organism suspension and 20 µl plant extract/antibiotic). Three control wells were maintained for each test batch. The positive control (antibiotic, Mueller-Hinton broth and test organism) and sterility control (Mueller-Hinton broth and DMSO) and organism control (Mueller-Hinton broth, test organism and DMSO). Plates were then incubated at 37° for 24 h overnight**.** Experiments were performed in triplicate. After incubation, 40 µl of 2-(4-iodophenyl)-3-(4-nitrophenyl) 5-phenyltetrazolium chloride (INT, Himedia, India) solution (0.2 mg/ml) dissolved in sterile distilled water was added to each well[25]. The plates were incubated for further 30 min, and estimated visually for any change in colour to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained clear corresponded to the MIC.

Determination of minimum bactericidal concentration:

MBC was determined from all wells showing no growth as well as from the lowest concentration showing growth in the MIC assay for all the samples. Bacterial cells from the MIC test plate were sub-cultured on freshly prepared solid nutrient agar by making streaks on the surface of the agar. The plates were incubated at 37° for 24 h overnight. Plates that did not show growth were considered to be the MBC for the extract or drug used[26]. The experiment was carried out in triplicate.

Determination of MIC index and statistical analyses:

The MIC index (MBC/MIC) was calculated for each extract and positive control drug to determine whether an extract had bactericidal (MBC/MIC $\leq$4) or bacteriostatic (>4 MBC/MIC <32) effect on growth of bacteria[27]. All experiments were repeated at least three times. Results are reported as mean±SEM.

RESULTS

The extractive yield varied among different parts of *M. charantia* and also among different hydroalcoholic extracts (hexane, 100, 75 and 25% methanol and water) as shown in (fig. 1). The hexane extract had very negligible yield in all the four parts of *M. charantia*. The areal part aqueous extract had slightly more extractive yield that 100% MeOH. As the concentration of methanol decreased, there was a slight increase in extractive yield (fig. 1a). The peel aqueous extract had considerably more extractive yield than 100% MeOH. As the concentration of methanol decreased, the extractive yield increased almost reaching to that of pure aqueous extract (fig. 1b). The extractive yield of hydroalcoholic extracts of pulp showed a trend similar to that of peel (fig. 1c). Both these parts, that is peel and pulp had maximum extractive yield. In seed also, pure methanol had considerable less extractive yield than aqueous extract;

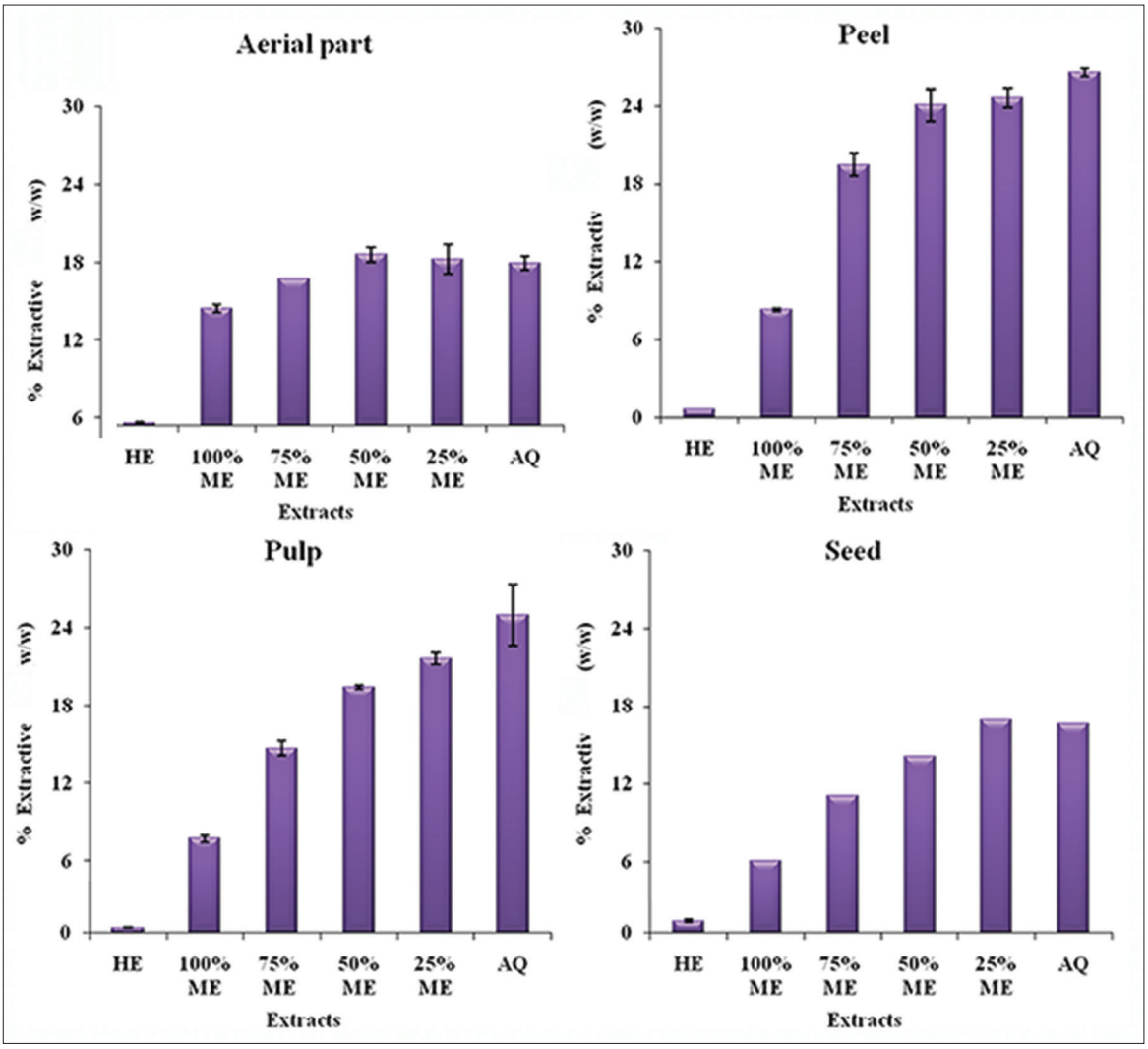

Fig. 1: Extractive yield of different solvent extracts of different parts of *M. charantia*.

the extractive yield of other hydroalcoholic extracts was similar to that of aerial parts (fig. 1d).

Antimicrobial activity of *Momordica charantia* aerial part:

The antimicrobial activity of different hydroalcoholic extracts of aerial part of *M. charantia* is shown in Table 1 and 2. All the extracts showed activity against *M. flavus* and *S. aureus*. The highest activity was in 50% MeOH followed by hexane extract against *M. flavus*. In Gram-negative bacteria, all the extracts showed activity against *P. syrigae* and *P. testosterone,* except *P. stutzeri.* The hexane extract showed maximum activity against *Pseudomonas* spp. All extracts showed moderate activity against fungi.

Antimicrobial activity of *Momordica charantia* peel:

The antimicrobial activity of different hydroalcoholic extracts of peel part of *M. charantia* is shown in Table 1 and 2. In Gram-positive bacteria, all extracts showed activity against *M. flavus* and the highest activity was in 50% MeOH extract. *S. albus* was resistant to all the extracts. In Gram-negative bacteria, all extracts showed activity against *P. syrigae, P. testosteroni* and *P. putida*; while remaining extracts showed different levels of activity against *P. aeruginosa, P. stutzeri and P. pictorum*. The 100 and 50% MeOH extracts showed activity against all *Pseudomonas* spp. screened. All extracts showed moderate activity against the fungi.

Antimicrobial activity of *Momordica charantia* pulp:

Antimicrobial activity of different hydroalcoholic extracts of the pulp of *Momordica charantia* is shown in Table 1 and 2. In Gram-positive bacteria, all extracts showed activity against *M. flavus* and the highest activity was in 75% MeOH extract followed by the hexane extract. *C. rubrum* and *S. albus* were

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT PARTS OF *M. CHARANTIA*

Part name	Extracts	Zone of Inhibition (mm)*										
		Gram positive bacteria					Gram negative bacteria (*Pseudomonas* Spp.)					
		CR	*SAL*	*SA*	*LM*	*MF*	*PA*	*PSt*	*PPi*	*PP*	*PT*	*PS*
Aerial part	HE	0±0	0±0	11±0	0±0	23±0	0±0	0±0	0±0	21.5±0.29	15.5±0.29	21.5±0.29
	MeOH	0±0	11±0	14±0	0±0	20.5±0	14±0	0±0	15±0	15±0	14±0	20±0
	75% MeOH	10±0	0±0	15±0	13.5±0.29	21±0	14±0	0±0	16±0	0±0	15±0	20±0
	50% MeOH	11±0	0±0	15±0	13±0	24±0	15±0	0±0	15.5±0.29	0±0	14.5±0.29	14.5±0.29
	25% MeOH	0±0	0±0	12±0	0±0	13±0	11±0	0±0	11±0	0±0	14.5±0.29	15.5±0.29
	AQ	0±0	0±0	10±0	0±0	10.5±0.29	0±0	0±0	0±0	16.5±0.29	15.5±0.29	17.5±0.29
Peel	HE	0±0	0±0	11±0	0±0	25±0	0±0	0±0	0±0	15±0	13.5±0.29	14.5±0.29
	MeOH	10±0	0±0	15±0	12±0	25±0	14.5±0.29	10.5±0.29	15±0	11.5±0.29	14.5±0.29	15.5±0.29
	75% MeOH	9±0	0±0	13.5±0.29	11.5±0.29	25±0	13.5±0.29	11±0	13±0	11.5±0.29	14.5±0.29	11.5±0.29
	50% MeOH	0±0	0±0	14.5±0.29	11±0	26.5±0.29	12±0	0±0	12.5±0.29	11.5±0.29	14.5±0.29	11.5±0.29
	25% MeOH	0±0	0±0	12±0	0±0	14±0	11.5±0.29	11±0	11±0	15±0	14.5±0.29	10±0
	AQ	0±0	0±0	0±0	0±0	14.5±0.29	0±0	10.5±0.29	10±0	10.5±0.29	13±0	14.5±0.29
Pulp	HE	0±0	0±0	0±0	0±0	21±0	11±0	0±0	0±0	14.5±0.29	16±0	19.5±0.29
	MeOH	0±0	0±0	10.5±0.29	0±0	20.5±0.29	12.5±0.29	10±0	16±0	17.5±0.29	15±0	14.5±0.29
	75% MeOH	0±0	0±0	11±0	0±0	23±0	13±0	10.5±0.29	13.5±0.29	14.5±0.29	16±0	16±0
	50% MeOH	0±0	0±0	11.5±0.29	10±0	20.5±0.29	12±0	0±0	12.5±0.29	13.5±0.29	15.5±0.29	0±0
	25% MeOH	0±0	0±0	0±0	0±0	20±0	10.5±0	0±0	0±0	12±0	14.5±0.29	0±0
	AQ	0±0	0±0	0±0	0±0	16±0	11.5±0	11.5±0.29	0±0	15±0	15±0	0±0
Seed	HE	10±0	0±0	9±0	0±0	25±0	10.5±0	11±0	10±0	13.5±0.29	10±0	14.5±0.29
	MeOH	10±0	0±0	10±0	11.5±0.29	16±0	12±0	10.5±0.29	11±0	17.5±0.29	10±0	20.5±0.29
	75% MeOH	9±0	12±0	10.5±0.29	11±0	18.5±0.29	10.5±0	11±0	11±0	13±0	10.5±0.29	16.5±0.29
	50% MeOH	10±0	11.5±0.29	11±0	12±0	18.5±0.29	11.5±0	11±0	11±0	15±0	10.5±0.29	15±0.29
	25% MeOH	0±0	12±0	11.5±0.29	0±0	19±0	0±0	11.5±0.29	0±0	16.5±0	10.5±0.58	14.5±0.29
	AQ	0±0	10.5±0.29	17±0	0±0	18±0	0±0	11±0	0±0	15±0	10±0.29	15.5±0.29

* The values are Mean±SEM (n=3)

TABLE 2: ANTIFUNGAL ACTIVITY OF DIFFERENT PARTS OF *M. CHARANTIA*

Part name	Extracts	Zone of Inhibition (mm)*			
		Fungal Strains			
		CE	*CA*	*CG*	*CN*
Aerial part	HE	11±0	15±0	13±0	11±0
	MeOH	12±0	14±0	11.5±0.29	12±0
	75% MeOH	10.5±0.29	13.5±0.29	11.5±0.29	10.5±0.29
	50% MeOH	9.5±0.29	11.5±0.29	10±0	9.5±0.29
	25% MeOH	0±0	14±0	11±0	0±0
	AQ	11.5±0.29	12.5±0.29	10.5±0.29	11.5±0.29
Peel	HE	10.5±0.29	11.5±0.29	11±0	10.5±0.29
	MeOH	12±0	12±0	10.5±0.29	12±0
	75% MeOH	10.5±0.29	12.5±0.29	10±0	10.5±0.29
	50% MeOH	10±0	13.5±0.29	11.5±0.29	10±0
	25% MeOH	11±0	13.5±0.29	12±0	11±0
	AQ	10±0	13±0	10.5±0.29	10±0
Pulp	HE	14±0	12.5±0.29	11±0	14±0
	MeOH	14±0	14±0	10±0	14±0
	75% MeOH	11.5±0.29	14±0	10.5±0.29	11.5±0.29
	50% MeOH	14.5±0.29	13.5±0.29	11±0	14.5±0.29
	25% MeOH	13±0	13±0	10.5±0.29	13±0
	AQ	10±0	13±0	10±0	10±0
Seed	HE	11.5±0.29	11.5±0.29	10±0	11.5±0.29
	MeOH	10.5±0.29	14±0	10.5±0.29	10.5±0.29
	75% MeOH	10±0	13±0	10.5±0.29	10±0
	50% MeOH	11±0	12±0	12±0	11±0
	25% MeOH	10±0	12.5±0.29	10±0	10±0
	AQ	0±0	0±0	9.5±0.29	0±0

resistant to all extracts. *L. monocytogenes* was slightly susceptible to only 50% MeOH extract. In Gram-negative bacteria, all extracts showed activity against *P. aeruginosa*, *P. testosteroni* and *P. putida*; while remaining extracts showed different levels of activity against *P. syrigae* and *P. pictorum.* The hexane extract showed maximum activity against *P. stutzeri.* All extracts showed moderate activity against the fungi.

Antimicrobial activity of *Momordica charantia* seed:

Antimicrobial activity of different hydroalcoholic extracts of seed part of *M. charantia* is shown in Table 1 and 2. In Gram-positive bacteria, all the extracts showed activity against *M. flavus* and *S. aureus* but susceptibility of *M. flavus* was considerably more than that of *S. aureus*. The highest activity was shown by the hexane extract against *M. flavus.* In Gram-negative bacteria, all the extracts showed activity against *P. testosteroni, P. putida, P. stutzeri* and *P. syrigae*; while *P. aeruginosa* and *P. pictorum* were not susceptible to any of the hydroalcoholic extracts. The highest activity was shown by 100% MeOH extract against *P. aeruginosa*. All extracts showed moderate activity against fungi.

All 24 extracts were compared with 5 standard antibiotics. These antibiotics were tested against 15 medically important microbial strains, the results of which were presented in Table 3. The antimicrobial activity of some of the hydroalcoholic extracts was comparable with that of standard antibiotics.

Determination of MIC, MBC and MIC index:

The MIC and MBC values of different hydroalcoholic extracts of different parts of *M. charantia* and standard antibiotics are shown in Tables 4-7. Inhibitory effects of bacterial growth by the extracts from different parts were in the range from <39 to >1250 µg/ml expressed as MIC values and in the range from 625 to >1250 µg/ml expressed as MBC values. Inhibitory effects of bacterial growth by the standard antibiotics were in the range from 4 to >32 µg/ml expressed as MIC values and in the range from 16 to >32µg/ml expressed as MBC values.

MIC and MBC values of different hydroalcoholic extracts of aerial part of *M. charantia* and standard antibiotics are shown in Table 4. Extract of 75% MeOH showed least MIC value and MBC values, that is 156 and 1250 µg/ml, respectively against *M. flavus*. However 100 and 75% MeOH extracts showed MIC index of 4; therefore both extracts showed bactericidal effect; it is comparable to standard chloramphenicol. Extract of 75% MeOH showed bactericidal effect against *P. pictorum*.

MIC and MBC values of different hydroalcoholic extracts of *M. charantia* pulp and standard antibiotics are shown in Table 5. Extracts of 75 and 100% MeOH showed least MIC values 312 and

TABLE 3: ANTIMICROBIAL ACTIVITY USING STANDARD ANTIBIOTICS

Antibiotic name	Concentration	Gram positive					Gram negative	Fungi			
		LM	*SAL*	*MF*	*SA*	*CR*	*PA*	*CE*	*CA*	*CG*	*CN*
Ampicilin	10 µg	0	0	0	23	25	14	NT	NT	NT	NT
Tetracycline	30 µg	0	21	0	22	0	0	NT	NT	NT	NT
Chloramphenicol	30 µg	0	26	0	18	15	0	NT	NT	NT	NT
Nystatin	100 units	NT	NT	NT	NT	NT	NT	22	18	18	22
Amphotericin	100 units	NT	NT	NT	NT	NT	NT	16	10	10	11

NT=Not tested, *SAL=Staphylococcus albus, CR=Corynebacterium rubrum, SA=Staphylococcus aureus, LM=Listeria monocytogenes, MF=Micrococcus flavus, CA=Candida albicans, CE=Candida epicola, CG=Candida glabrata, CN=Candida neoformans, PA=Pseudomonas aeruginosa*

TABLE 4: MIC AND MBC OF DIFFERENT SOLVENT EXTRACTS OF *M. CHARANTIA* AERIAL PARTS

Extracts	*MF*[1]			*PPi*[2]			*PP*[2]			*PT*[2]			*PS*[2]		
	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index
HE	>1250	>1250	ND	-	-	-	>1250	>1250	ND	>1250	>1250	ND	>1250	>1250	ND
100% MeOH	312	1250	4.0	-	-	-	-	-	-	-	-	-	>1250	>1250	ND
75% MeOH	156	625	4.0	312	1250	4.0	-	-	-	-	-	-	>1250	>1250	ND
50% MeOH	625	>1250	ND	>1250	>1250	ND	-	-	-	-	-	-	-	-	-
25% MeOH	-	-	-	-	-	-	-	-	-	--	-	-	-	-	-
AQ	-	-	-	-	-	-	>1250	>1250	ND	>1250	>1250	ND	>1250	>1250	ND
CH	4	16	4	32	>32	ND	16	>32	ND	32	>32	ND	16	>32	ND
CF	16	32	2	8	>32	ND	32	>32	ND	8	32	4.0	>32	>32	ND

MIC is minimum inhibitory concentration and MBC is minimum bactericidal concentration. -: Not tested, 1: Gram-positive bacteria, 2: Gram-negative bacteria, ND=not determined, *MF=Micrococcus flavus, PPi=Pseudomonas pictorum, PP=Pseudomonas putida, PT=Pseudomonas testosteroni, PS=Pseudomonas syrigae,* HE=hexane extract, CH=chloramphenicol, CF=ceftazidime

TABLE 5: MIC AND MBC OF DIFFERENT SOLVENT EXTRACTS OF *M. CHARANTIA* PULP

Extracts	*MF*[1]			*PP*[2]			*PT*[2]			*PS*[2]		
	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index
HE	625	>1250	ND	-	-		>1250	>1250	ND	>1250	>1250	ND
100% MeOH	156	1250	8.0	625	>1250	ND	-	-	ND	-	-	ND
75% MeOH	312	1250	4.0	-	-	ND	>1250	>1250	ND	-	-	-
50% MeOH	625	>1250	ND	-	-	-	>1250	>1250	ND	-	-	-
25% MeOH	1250	>1250	ND	-	-	-	-	-	-	-	-	-
AQ	>1250	>1250	ND	-	-	-	-	-	-	-	-	-
CH	4	16	4	16	>32	ND	32	>32	ND	16	>32	ND
CF	16	32	2	32	>32	ND	8	32	ND	>32	>32	ND

MIC is minimum inhibitory concentration and MBC is minimum bactericidal concentration. -: Not tested, 1: Gram-positive bacteria, 2: Gram-negative bacteria; ND=not determined, *MF=Micrococcus flavus, PP=Pseudomonas putida, PT=Pseudomonas testosteroni, PS=Pseudomonas syrigae,* HE=hexane extract, CH=chloramphenicol, CF=ceftazidime

156 μg/ml, respectively, and MBC was 1250 μg/ml against *M. flavus*. Extracts of 100% and 75% MeOH showed MIC index of 8 and 4, respectively; therefore, 100 and 75% MeOH showed bactericidal and bacteriostatic effect against *M. flavus*. Extracts of 100% MeOH showed MIC value of 625 μg/ml while MBC was >1250 μg/ml against *P. putida*.

MIC and MBC values of different hydroalcoholic extracts of *M. charantia* peel and standard antibiotics are shown in Table 6. Extracts with 50% and 100% MeOH showed least MIC values, 312 and 156 μg/ml, respectively, and MBC was 1250 and 625 μg/ml, respectively, against *M. flavus*. Both extracts showed bactericidal effect.

MIC and MBC values of different hydroalcoholic extracts of *M. charantia* seed and standard antibiotics are shown in Table 7. MeOH extracts (50 and 100%) showed least MIC values <39 μg/ml and MBC was 625 μg/ml against *P. putida*. Remaining extracts showed >1250 μg/ml MIC and MBC values. In this study, bactericidal effect was shown by 100, 75 and 50% MeOH extracts against *M. flavus* and *P. pictorum* while remaining extracts showed bacteriostatic effects.

TABLE 6: MIC AND MBC OF DIFFERENT SOLVENT EXTRACTS OF *M. CHARANTIA* PEEL

Extracts	*MF*[1]			*PS*[2]		
	MIC	MBC	MIC index	MIC	MBC	MIC index
HE	>1250	>1250	ND	-	-	-
100% MeOH	156	625	4	>1250	>1250	ND
75% MeOH	-	-	-	-	-	-
50% MeOH	312	1250	4	-	-	-
25% MeOH	-	-	-	-	-	-
AQ	-	-	-	>1250	>1250	
CH	4	16	4	16	>32	ND
CF	16	32	2	>32	>32	ND

MIC is minimum inhibitory concentration and MBC is minimum bactericidal concentration. -: Not tested, 1: Gram-positive bacteria, 2: Gram-negative bacteria, ND=not determined, *MF=Micrococcus flavus*, *PS=Pseudomonas syrigae*, HE=hexane extract, CH=chloramphenicol, CF=ceftazidime

DISCUSSION

Normally, a high extraction yield is required for an efficient process; although it is not necessary that high concentration of bioactive components are present in them. Since some bioactive components are very sensitive to oxygen and heat[28], care should be taken to prevent their oxidation and thermal degradation. Therefore, the extraction yield and the bioactive component characteristics should also be considered when an extraction method is selected. The traditional healers or practitioners make use of water primarily as a solvent but there are many reports where organic solvents showed better activity as compared with aqueous extracts[29-31]. In the present study, extractive yield was considerably more in water than in organic solvent methanol and as the concentration of methanol decreased, extractive yield increased clearly indicating that in these plant parts, water soluble phytoconstituents were more.

The results of antimicrobial activity clearly indicated that *M. flavus* was the most susceptible Gram-positive bacteria and *P. testosterone* was the most susceptible Gram-negative bacteria. Aqueous extract showed poor activity as compared with pure methanol or hydroalchoholic extracts; best antibacterial activity was shown by 100% MeOH extract, which is an extract with a pure organic solvent. Almost all the extracts showed antifungal activity against all the four fungi studied, though the activity was moderate.

Gram-negative bacteria were more susceptible towards all the extracts than Gram-positive bacteria. This is very good report since there is a general

TABLE 7: MIC AND MBC OF DIFFERENT SOLVENT EXTRACTS OF SEEDS OF *M. CHARANTIA*

Extracts	*MF*[1]			*PPi*[2]			*PP*[2]			*PT*[2]			*PS*[2]		
	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index	MIC	MBC	MIC index
HE	>1250	>1250	ND	-	-	-	-	-	-	-	-		-	-	
100% MeOH	-	-	-	-	-	-	<39	625	ND	-	-		>1250	>1250	ND
75% MeOH	>1250	>1250	ND	-	-	-	-	-	-	-	-		>1250	>1250	ND
50% MeOH	>1250	>1250	ND	-	-	-	<39	625	ND	-	-		-	-	
25% MeOH	>1250	>1250	ND	-	-	-	>1250	>1250	ND	-	-		-	-	
AQ	>1250	>1250	ND	-	-	-	-	-	-	-	-		>1250	>1250	ND
CH	4	16	4	32	>32	ND	16	>32	ND	32	>32	ND	16	>32	ND
CF	16	32	2	8	>32	ND	32	>32	ND	8	32	4	>32	>32	ND

MIC is minimum inhibitory concentration and MBC is minimum bactericidal concentration. -: Not tested, 1: Gram-positive bacteria, 2: Gram-negative bacteria, ND=not determined, *MF=Micrococcus flavus*, *PPi=Pseudomonas pictorum*, *PP=Pseudomonas putida*, *PT=Pseudomonas testosteroni*, *PS=Pseudomonas syrigae*, HE=hexane extract, CH=chloramphenicol, CF=ceftazidime

consensus that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria[32-40]. Therefore, the search is always to find plant extracts that are capable of inhibiting Gram-negative bacteria, which are equally dangerous in causing infectious diseases like Gram-positive bacteria. The Gram-negative cell wall (made up of lipopolysaccharide) is complex and multilayered structure, which makes access to membrane more restricted and barrier to many environmental substances including synthetic and natural antibiotics. The results of the present study indicate that extracts of *M. charantia* contain some secondary metabolites, which are able to cross this tough barrier.

The MIC is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation[41,42], whereas the MBC is interpreted as the lowest concentration that can completely remove the microorganisms. A pinkish coloration is indicative of microbial growth because of their ability to convert INT to red formazan[43]. The concentrations of MIC and MBC for plant extracts and standard antibiotics were 1250–39 µg/ml and 32–1 µg/ml, respectively. MIC and MBC were expressed in terms of µg/ml. Braca *et al.*[44], Coutinho *et al.*[45] and Roopashree *et al.*[46] used in their studies *M. charantia* extracts/essential oils, which possessed potential activity against *Staphylococcus aureus*. Castilho *et al.*[47] reported that antimicrobial activity of *Origanum* essential oils and all the studied extracts showed MIC values >200 µg/ml against *P. aerugenosa*. In the present study, better results were found in seed (100% methanol and 50% methanol) and MIC value was <39 µg/ml, which was near to ceftazidime against *P. pictorum*.

The spread of multidrug-resistant strains of microorganisms and the reduced number of drugs available makes it necessary to discover new classes of antibacterial and antifungal agents that overcome these resistant mechanisms. This led to search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used empirically for their antibacterial and antifungal properties.

Foodborne disease is one of the major concerns to food producers and consumers and spoilage of foods is still a major problem in different parts of the world. In an effort to meet this demand, the food industry has a great interest in using natural antimicrobial compounds. The hydroalcoholic extracts of *M. charantia* possessed significant antibacterial activity (MIC ≤39 µg/ml) against *Pseudomonas* spp. Therefore, the use of this plant as antimicrobial agent is validated by the results obtained in this work. Further studies are in progress to identify the chemical compounds present in these extracts with antimicrobial activity as well as to identify synergism between plant extracts and standard antibiotics. The results of the present investigation also provide an approach to develop promising natural antimicrobial agents with potential applications in the food and pharmaceutical industries. This fact is of paramount importance from the point of view of food safety.

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