ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Phytotoxicity of *Momordica Charantia* Extracts against *Alternaria Alternata*

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Abstract

Mycobiocides are attracting research interest worldwide as possible plant pathogens control measures to replace synthetic fungicides. In this study, the application of plant part extracts leaves, stem, fruits, and root of *Momordica charantia* as fungicides were evaluated. The *in vitro* antifungal effects of aqueous and ethanolic extracts of the plant materials of *M. charantia* to inhibit the fungal mycelial growth (diameter) and percent spore germination of *A. alternata* has been evaluated by poison food technique and spore germination method. All the ethanolic extracts exhibited varying degree of percent inhibition in spore germination and mycelial growth. Ethanolic extracts of stem and leaves of *M. charantia* showed hundred percent inhibitions in spore germination of *A. alternata* at 60mg/ml conc. At 50% concentration strong inhibition of spore germination of test fungi was recorded in ethanolic extract of fruit (86.11±2.40) and root (86.36±0.00) followed by leaves (69.44±2.40) and stem (62.87±1.31). Against mycelial growth, strong inhibition was recorded in ethanolic extract of stem (79.04±1.06%) and leaves (73.55±2.40%) trailed by root (44.20±0.71%) and fruit (42.3±1.70%). Aqueous extracts of all parts of the plant showed comparatively less significant amount of inhibition in spore germination and mycelial growth. Ethanolic extracts of leaves showed 1.25 mg/ml MIC value against *A. alternata* followed by stem (2.5 mg/ml), fruit (10 mg/ml), and root (20 mg/ml). All the dried powdered samples of the plant parts of *M. charantia* were analyzed for the quantitative estimation of phytoconstituents (phenolics, tannins, flavonoids and saponins). These compounds may be responsible for activity in extracts of different plant parts.

Key Words: *Momordica*, Fungi, Extracts, *Alternaria*

INTRODUCTION

Fungal secondary metabolites produced by some phytopathogenic spoilage fungi such as *Alternaria* species that are hazardous for consumers' health, and lead to economic losses of commercial value of food[1]. *Alternaria* species is ubiquitous, and found worldwide in association with a wide variety of substrates. Many species are saprophytes but most are known for their impact as plant and animal pathogens[2, 3]. *Alternaria* have a wide host range as plant pathogens, ranking 10th in terms of total number of plant hosts[4]. It is widespread and of great economic importance because it causes destructive leaf spots, foliar and blossom blight, blemishes and damage to a great variety of fruits and seeds from numerous hosts. It represents about 4% of the fungal diversity but cause 80% of foliar losses in some parts of the world[5]. *Alternaria* sp. represents one of the most important post-harvest pathogens[6] contributes to extensive losses of our agricultural output due to spoilage[7]. In addition, they are one of the most common airborne allergens, as well as being one causative agent of phaeohyphomycosis in immunocompromised patients[2].

Fungal diseases have been one of the most important causes of crop losses ever since humans started to cultivate plants (Harvey, 1978). At least 20% of agricultural spoilage is caused by *Alternaria* species; most severe losses may reach up to 80% of yield[8]. Plants are endowed with several defense mechanisms that protect them from fungal infection. Physical contact of the fungal pathogens on plant cell surface results in activation of host defense mechanism. This includes an induction of genes encoding enzymes such as phenylalanine ammonia-lyase, 4 coumarate-CoA ligase, cinnamic acid-4- hydroxylase, cinnamyl alcohol dehydrogenase, chalcone synthase, chalcone isomerase, among others, which are involved in synthesis of phytoalexins, phenols, lignins, tannins, and melanins with antifungal activity[9]. The indiscriminate use of synthetic agrochemicals to control agricultural fungal infections has led to the development of resistance in phytopathogenic fungal populations, which is one of the most important problems in agriculture[10]. A good alternative to chemical fungicides utilization in fungal disease control is plant extracts.

Momordica charantia Linn, belongs to the Cucurbitaceae family, is a medicinal vegetable that grows in the tropical and subtropical parts of the world. It is commonly known as ‘‘bitter melon’’ or ‘‘bitter gourd’’ because all parts of the plant, including the fruit, taste bitter[11, 12]. Leaves, stem, fruit and root of *M. charantia* extracts contains several biologically active compounds, chiefly momordicin I and momordicin II, and cucurbitacin B. The main constituents of bitter melon (Karela) are triterpene, protein, steroid, alkaloid, glycosides, saponins, lipid, phenolic compounds, ascorbic acid, and free amino acids[11, 13]. Various studies proved that phytochonstituents of the plant extracts may be responsible for their antifungal activity[14-18]. Recent scientific evidence has verified that M. *charantia* extracts and essential oils is a promising antifungal agent[19, 20]. Based on the antifungal activity, crude plant extracts may be a cost effective way of protecting crops against fungal pathogens. Because plant extracts contain several antifungal compounds, the development of resistant pathogens may be delayed.

The present study was aimed to evaluate the fungitoxic potential of leaves, stem, fruits and roots *M. charantia*

against plant pathogenic / food spoilage fungi i.e. *Alternaria alternata*.

MATERIALS AND METHODS

Plant Materials

Leaf, stem and roots of *M. charantia* were collected from the plants grown in the botanical garden of Jiwaji University, Gwalior (M.P.) while the fruits were obtained from the local market of Gwalior (M.P.). Collected samples were washed with tap water followed by sterilized distilled water. Samples were dried in hot air oven at 60°C and homogenized to fine powder. Powdered samples were stored in airtight container at room temperature for further study.

Extraction Procedure

(i) Aqueous extract

50 g sample powder was immersed in 200 ml of hot distilled water and kept in a water bath for 20 min at 80-85°C, removed and percolated for 24 hrs. These extracts were filtered through Whatman filter paper (No-1) and the filtrate was centrifuged at 5000 rpm for 20 minutes. The supernatant was then evaporated and the crude extracts were assayed against the test organism[21].

(ii) Ethanolic extract

10g sample powder was extracted with 100 ml of ethanol (80%) for 12 hrs using a Soxhlet extractor, obtained extract was evaporated to dryness and assayed against the test organism[22].

Test Organism and Preparation of Spore Suspension

A. alternata (Fr.) Keissl. (ITCC # 6306) was obtained from the Indian Type Culture Collection Centre, IARI, New Delhi. Fungal strain was maintained on the Potato Dextrose Agar (PDA) media at 4° C. Conidial suspension of *A. alternata* was prepared from their respective 7 day old cultures by mixing the fungal mycelial plugs with sterile distilled water followed by filtration through glass wool. The spore concentration was adjusted to 1×10^8 spores/ml for fungus using haemocytometer[23].

Fungitoxicity Assay

Fungitoxic activity of aqueous and ethanolic extracts of all parts of *M. charantia* was evaluated on the basis of percent inhibition of mycelial growth by Poisoned food technique[24] and percent inhibition of germination of spores[25] followed by determination of minimum inhibitory concentration[26].

Poisoned food technique

Aqueous and ethanolic extracts of leaves, stem, fruit and root of *M. charantia* was prepared by re-dissolving the crude extracts separately in 100ml of sterilized distilled water. Different concentrations (10, 20, 30, 40, and 50%) of aqueous and ethanolic extracts were subjected to fungitoxic assay. To get the required concentrations (10, 20, 30, 40, and 50%), plant extracts were added in a specific amount in PDA. Total 20 ml medium was poured into sterilized Petri dishes and allowed to solidify.

After complete solidification of the medium, a disc (5 mm in diameter) was removed from 7-day old culture of the test fungi using a cork borer and then transferred upside down into the center of each Petri plate containing different concentrations of the extracts. Five replicates were maintained for each concentration and Petri plates of PDA medium without extracts served as control. The plates were sealed with paraffin wax and incubated at 25±1ºC for 8 days.

After incubation, the diameter of fungal colony was measured in mm at 4th, 6th and 8th day of incubation and the fungitoxicity of the extracts in term of percentage inhibition of mycelial growth was calculated.

Percent spore germination assay

The aqueous extract of leaves, stem, fruit, and root was prepared by re-dissolving 100 mg crude extract in 1 ml of sterilized distilled water whereas the ethanolic extracts was prepared by re-dissolving 100 mg crude extract in 1 ml of 10% dimethyl sulfoxide (DMSO). Conidial suspension of test fungi was prepared in sterilized distilled water and spore concentration was adjusted to 1×10^8 spores/ml. Five concentrations of aqueous and ethanolic extracts (10, 20, 30, 40 and 50mg/ml) and one control without plant extracts (10% DMSO with sterile distilled water for ethanolic extract) were separately tested for spore germination of *A. alternata.*

The conidial suspension mixed with specific concentrations of the aqueous or ethanolic extract was taken in an Eppendorf tube. Controls without extracts were also maintained. The tubes were incubated at 25± 1°C for 18 hours. After 18 hours the test solution was placed in both chambers of a haemocytometer by carefully touching the edges of the cover slip with the pipette tip and allowed to fill the counting chamber. Spore germination was counted under the compound microscope by using haemocytometer cell counting method. All the experiments were conducted in triplicate.

MIC (Minimum Inhibitory Concentration)

The minimum inhibitory concentration (MIC) of the aqueous and ethanolic extracts of leaves, stem, fruits, and root of *M. charantia* was determined by two-fold dilution method. Crude extracts of the all parts of *M. charantia* was dissolved in hot water (for aqueous extracts) and in 10% DMSO (for ethanolic extract) respectively. These extracts were serially diluted and 200 µl of the extract was added to a 700µl of potato dextrose broth (PDB in test tubes to make the final concentrations of 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 mg/ml). 100 µl spore suspension of test strain was inoculated in the test tubes containing PDB and extract and incubated for 2 to 7 days at 25±1°C. The control tubes containing PDB medium only were inoculated with fungal spore suspensions. The concentrations at which visible inhibition of fungal mycelium was observed treated as the MIC (mg/ml).

CALCULATIONS

Fungitoxicity of the extracts in term of percent inhibition of mycelial growth and percent inhibition of germinated spores was calculated using the formula as described by Deans and Svoboda (1990)[27].

$$\% \text{ inhibition} = [(C - T) / C \times 100]$$

Where,

C = is the colony diameter of the mycelium on the control dish (mm) or number of spores germinated in control

T = is the colony diameter of the mycelium on treatment dish (mm) or number of spores germinated in treatment.

PHYTOCHEMICAL ANALYSIS

The dried powder of leaves, stem, fruits and roots of *M. charantia* was subjected to phytochemical analysis to quantify the alkaloids (Harborne, 1973)[28], flavonoids (Zhuang et al., 1992)[29], total phenolics and tannins (Makkar et al., 1993)[30] and saponins (Obadoni and Ochuko, 2001)[31].

STATISTICAL ANALYSIS

Statistical analysis was performed using Minitab software: version 16.0. The results are presented as the mean ± S.D. One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to check the significance of the results with the level of significance set at $p<0.05$.

RESULTS

Inhibitory Effect of Plant Extracts on the Mycelial Growth of *A. alternata*

Various concentrations (10, 20, 30, 40 and 50%) of aqueous and ethanolic extracts of leaves, stem, fruit and root of *M. charantia* have been tested for their antifungal potential against the growth of *A. alternata*. Observations were recorded at various intervals i.e. on 4th, 6th, and 8th day of incubation period.

Effect of the ethanolic extracts on A. alternata

The effect of various conc. of the ethanolic extracts of leaves, stem, fruits, and roots of *M. charantia* is shown in table 1. According to the ANOVA on the mycelial growth of *A. alternata*, there were highly significant differences ($P\leq0.01$), on the fruits and roots extracts. Ethanolic extracts of all plant parts were found effective in inhibiting the mycelial growth of *A. alternata* at 50% concentration after 8th days. Among the plant parts, stem and leaves extract exhibited highest inhibition i.e. 79.04±1.06% and 73.55±2.40%, respectively followed by root (44.20±0.71%) and fruit (42.3±1.70%) against the tested fungi.

Effect of the aqueous extracts on A. alternata

None of the extract (leaves, stem, fruit, and root of *M. charantia*) was found much effective in inhibiting the mycelial growth of *A. alternata* at all concentrations even after 8th days of incubation period (Table 1). Among all plant parts (leaves, stem, fruit and root), aqueous extracts of stem exhibited 25.23±1.65% inhibition in the mycelial growth of *A. alternata* followed by root (19.53±1.27%), leaves (19.04±1.68%) and fruit (4.76±1.68%) at 50% concentration after 8th days.

Inhibitory Effect of Plant Extracts on the Spore Germination of *A. alternata*

Effect of aqueous and ethanolic extracts of plant parts, on the spore germination of *A. alternata* and *F. oxysporum* are summarized in table 2. Di Methyl Sulfoxide (DMSO) 10% v/v used as a negative control did not inhibit the spore germination of the plant pathogen tested. There was a significant inhibition in spore germination of *A. alternata* by different concentrations of ethanolic extract. Ethanolic extracts of leaves, stem, fruits and root were found highly effective in inhibiting the spore germination of *A. alternata* at 50mg/ml concentration. Complete inhibition in spore germination of *A. alternata* was recorded at 60mg/ml concentration by fruit and root ethanolic extracts. Strong inhibition 86.11±2.40% and 86.36±0.00% was observed in spore germination of *A. alternata* in the ethanolic extract of fruit and root, respectively 50mg/ml concentration followed by leaves (69.44±2.40%) and stem (62.87±1.31%) at the same concentration.

Among aqueous extracts of plant parts, only root extract was found most effective (59.76±1.98%) in inhibiting the spore germination of *A. alternata* at 50mg/ml concentration followed by 53.70±3.20% (in fruit), 26.35±3.55% (leaves), and 23.68±2.63% (stem).

Table 1. Effect of aqueous and ethanolic extracts of leaves, stem, fruit, and roots of *M. charantia* on the mycelial growth of *A. alternata* after 8th day.

S.N.	Conc. (%)	Percent Inhibition (%)							
		Leaves		Stem		Fruit		Root	
		Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.
1.	10	0.00 ±0.00^{d}	51.92 ±1.70^{c}	3.73 ±1.04^{d}	45.23 ±0.84^{d}	1.42 ±2.12^{b}	9.13 ±1.61^{e}	6.97 ±0.82^{d}	7.36 ±0.72^{e}
2.	20	3.80 ±1.30^{c}	63.45 ±2.63^{b}	3.73 ±1.04^{d}	45.70 ±1.06^{d}	1.90 ±1.06^{ab}	14.42 ±1.31^{d}	10.69 ±1.27^{c}	23.68 ±2.63^{d}
3.	30	3.80 ±1.30^{c}	65.86 ±1.07^{b}	7.47 ±1.27^{c}	56.66 ±1.99^{c}	1.90 ±1.06^{ab}	22.11 ±1.31^{c}	13.01 ±1.27^{b}	32.62 ±1.44^{c}
4.	40	7.14 ±2.38^{b}	71.63 ±2.00^{a}	19.15 ±1.28^{b}	69.99 ±1.30^{b}	4.28 ±2.12^{ab}	36.83 ±1.97^{b}	17.66 ±1.27^{a}	37.36 ±1.17^{b}
5.	50	19.04 ±1.68^{a}	73.55 ±2.40^{a}	25.23 ±1.65^{a}	79.04 ±1.06^{a}	4.76 ±1.68^{b}	42.3 ±1.70^{a}	19.53 ±1.27^{a}	44.20 ±0.71^{a}

Aq. Ext. – Aqueous extract; **Eth. Ext.**- Ethanolic extract

Values are given as mean ±S.D. of five replicates

Means in columns that do not share a superscript letter are significantly different (One way ANOVA at $P<0.05$ followed by Tukey HSD test).

Table 2. Effect of aqueous and ethanolic extracts of various plant parts of *M. charantia* on the spore germination of *A. alternata*.

S.N.	Conc. (mg/ ml)	Percent Inhibition (%)							
		Leaves		Stem		Fruit		Root	
		Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.	Aq. Ext.	Eth. Ext.
1.	10	10.00 ±1.25[b]	16.37 ±0.97[d]	13.44 ±2.67[b]	16.66 ±0.82[e]	28.06 ±1.51[b]	30.66 ±1.15[f]	9.94 ±1.72[d]	14.47 ±1.72[f]
2.	20	12.88 ±3.07[b]	22.96 ±1.28[c]	16.95 ±2.02[ab]	23.27 ±0.91[d]	34.34 ±1.74[b]	39.0 ±1.23[e]	14.47 ±1.72[d]	27.53 ±1.25[e]
3.	30	20.89 ±2.58[a]	33.32 ±1.55[b]	19.14 ±2.12[ab]	25.58 ±1.72[c]	47.12 ±1.99[a]	50.87 ±3.04[d]	25.53 ±2.51[c]	40.94 ±3.29[d]
4.	40	26.00 ±0.00[a]	37.49 ±3.12[b]	21.92 ±4.01[a]	41.37 ±1.72[b]	50.0 ±5.00[a]	67.77 ±3.85[c]	39.99 ±2.85[b]	62.06 ±0.00[c]
5.	50	26.35 ±3.55[a]	69.44 ±2.40[a]	23.68 ±2.63[a]	62.87 ±1.31[a]	53.70 ±3.20[a]	86.11 ±2.40[b]	59.76 ±1.98[a]	86.36 ±0.00[b]
6.	60	-	-	-	-	-	100.0 ±0.00[a]	-	100.0 ±0.00[a]

Aq. Ext. – Aqueous extract; **Eth. Ext.**- Ethanolic extract
Values are given as mean ±S.D. of five replicates
Means in columns that do not share a superscript letter are significantly different (One way ANOVA at P <0.05 followed by Tukey HSD test).

Minimum Inhibitory Concentrations (Mic) of Plant Part Extracts to Inhibit the Growth of *A. alternata*

The lowest concentration of the plant extracts that prevented the fungal growth was used to determine the MIC. The growth of test organism after 72 hours of incubation in the presence of different concentrations of extracts was compared to the control. Lowest MIC was recorded in ethanolic extracts as compared to aqueous extracts. Ethanolic extract of leaves showed 1.25 mg/ml MIC for *A. alternata* followed by stem (2.5mg/ml), fruits (10mg/ml), and roots (20mg/ml) (Table 3). No MIC (>40mg/ml) was recorded in aqueous extracts of fruit and root against *A. alternata* whereas the aqueous extracts of leaves and stem showed 10 and 20mg/ml MIC for *A. alternata* respectively.

Table 3. Minimum inhibitory concentration (MIC) of aqueous and ethanolic extracts of various plant parts of *M. charantia* against *A. alternata*.

S. N.	Plant parts	Minimum Inhibitory Concentration (mg/ml)	
		Aqueous Extract	Ethanolic extract
1.	Leaves	10	1.25
2.	Stem	10	2.5
3.	Fruits	>40	10
4.	Root	20	20

Phytochemical Analysis

During study various plant parts were found to possess a good number of secondary metabolites (Table-4). Among leves, stem, fruit and roots highest concentration of phenolics was recorded from fruit (4.280±0.56%) followed by stem (3.200±0.18%), leaves (0.880±0.06%) and roots (0.500±0.07). Fruits and stem were also found rich in tannin with concentrations 3.40±0.65 and 2.70±0.17% respectively. A good concentration of flavonoid was also recorded from leaves (0.632±0.10), stem (0.216±0.02) and fruits (0.131±0.01%). Saponin was also recorded from all the plant parts with varying concentrations.

Table 4. Quantitative estimation of phytoconstituents

S. N.	Phytoconstituents	Quantity of phytoconstituents (g/100g) on dry matter basis			
		Leaves	Stem	Fruit	Root
1.	Phenolics	0.880 ±0.06	3.200 ±0.18	4.280 ±0.56	0.500 ±0.07
2.	Tannins	ND	2.70 ±0.17	3.40 ±0.65	ND
3.	Flavonoids	0.632 ±0.10	0.216 ±0.02	0.131 ±0.01	ND
4.	Saponin	2.050 ±0.01	1.350 ±0.2	1.220 ±0.03	1.250 ±0.03

ND – Not detected

DISCUSSION

Antifungal active extracts and oils derived from plants are generally nonphytotoxic (Pandey et al., 1982)[32]. Thus plant extracts are promising natural antifungal agents. Active antifungal principles with strong inhibitory effects against *Aspergillus niger* and *Trichophyton rubrum*, detected from *Curcuma zedoaria*, *C. aromatic*, *C. amada* and *Brassica* species have already been reported by Gupta and Banarjee (1972)[33].

Among all the screened plant parts of *M. charantia*, the aqueous and ethanolic extracts of leaf exhibited moderate and strong antifungal activity respectively against the tested fungi. This is in agreement with the findings of Leelaprakash *et al*. (2011)[34], who also made the similar observations and reported that aqueous leaf extracts also have antimicrobial activity. Ethanolic leaf extract and essential oil of its seeds has also been reported to possess strong antimicrobial activity against the bacterial strain of *Staphylococcus aureus* (Coutinho *et al*., 2010)[35]. Mwambete (2009)[36] and Jagessar *et al*. (2010)[37] also reported antimicrobial activity of alcoholic extracts of leaf of *M. charantia.* Results from the present study could be correlated with the studies made by Mughal *et al*. (1996)[38] with leaf extracts from *Allium sativum*, *Datura alba* and *Withania somnifera* against *Alternaria alternata*, *A. brassicola* and *Myrothesium rodium*. Gupta et al., (2015)[39]

also reported the effectiveness of ethanolic leaf extract of *Calotropis procera* against the mycelial growth of *Alternaria alternata* at a concentration of 10mg/ml.

Burger *et al*. (2010) [40] reported comparatively good effects of ethanolic extracts of the leaves of *Momordica* species on spore germination of *A. solani* than the aqueous fractions of leaves. Similarly, ethanolic extracts of leaves of *M. charantia* was found effective in inhibiting the mycelial growth of *A. alternata*. This could be attributed to the fact that antifungal compounds present in the leaf extracts might have extracted well in these organic solvents than aqueous extract [41]. Leaves are rich in a wide variety of secondary metabolites such as glycosides, alkaloids, proteins, saponins and phytosterols which have been found *in vitro* to have antimicrobial properties[42]. This is further confirmed by the findings of the present investigations where leaves were found to possess various secondary metabolites i.e. saponin, phenolic and flavonoids. This result certainly indicates that ethanolic extracts contained higher concentrations of active antimicrobial agents than aqueous extracts and therefore showed higher fungitoxic activity than aqueous fractions.

During the investigation, aqueous extracts of stem of *M. charantia* significantly inhibited the mycelial growth of *A. alternata* indicating good antifungal potential of extract. Good antifungal potential of aqueous stem extracts of *Cymbopogon proximus* and *Zingiber officinale* against the growth of *A. alternata* has already been reported by Fawzi *et al*. (2009) [43]; the aqueous extract of stem bark of *syzygium cumini* showed potential to inhibit the growth of *A. alternata* [44].

We have not found any report related to the fungitoxic activity of stem of *M. charantia* in the available literature Therefore, in our view probably this is the first time that the good fungitoxic activity of stem extracts (aqueous and ethanolic) of *M. charantia* against the mycelial growth as well as spore germination of *A. alternata* has been reported. Although reports are available on the inhibition of spore germination of *A. alternata* by stem extracts of *Capparis deciduas*, *Lantana camara* and *Tridax procumbens* [16]. During investigation stem extract of *M. charantia* was also found rich in various secondary metabolites like phenolics, tannins, saponins and flavonoids. This might be the probable reason behind the fungistatic effect of stem extracts as these secondary metabolites have already been proved for their role in plant defense mechanism [45].

During the present investigation ethanolic fruit and root extracts of *M. charantia* was found strongly effective in inhibiting the spore germination of *A. alternata*. Fruit and root extract at the level 60% exhibited 100% inhibition against the spore germination of *A. alternata*. None of the earlier studies have reported 100% fungistatic activity of various plant parts of *M. charantia*, in the available literature. Feng and Zheng (2007)[46] have reported fungistatic action of *Cassia* oil even at lower concentration; they observed total inhibition of the radial growth of *A. alternata* by *Cassia* oil at 300 ppm. This high antifungal activity of ethanolic extracts of fruit might be due to the presence of high quantity and diverse types of the phytoconstituents in fruits as compared to the other parts of the plant[47,48]. This is further confirmed by the findings of the present investigations where fruit extracts was found to possess various medicinally valuable secondary metabolites. Stange *et al*. (1999)[49] isolated a phytoalexins from fruit tissue of *Cucurbita maxima* and reported it as an induced antifungal compound. Indeed, plants affiliated to the cucurbitaceae, including *Momordica charantia*, produce a number of proteins and peptides that are indicative of antifungal activity, including trypsin inhibitors, lectins [50], ribosome-inactivating proteins[51-55] and ribonucleases[56].

Antimicrobial resistance of fungal pathogens is a major problem for the treatment of plants with microbial diseases. To confirm the resistance of pathogen to an antimicrobial agent against the pathogens determination of Minimum inhibitory concentrations (MICs) of any antimicrobial agent is required. MIC of plant extracts for fungus plays a crucial role in the determination of antifungal resistance of fungi. According to Andrews (2001)[56], MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

During investigation leaves, stem, fruits and root extracts of *M. charantia* against *A. alternata* showed variation in their MIC values. However, MIC of ethanolic fruit extracts of *M. charantia* was 10mg/ml for *A. alternate* whereas Duru and Onyedineke (2010)[57] reported 100g/ml MIC against *Alternaria solani* by ethanolic seed extracts of *Voacanga africana*. Present investigation reveals that ethanolic leaf extracts of *M. charantia* showed 1.25 mg/ml MIC value for *A. alternata.* This is in agreement with the findings of Dellavalle *et al*. (2011)[58], where leaf extracts of *Salvia officinalis* showed MIC value 2.5µg/ml against *Alternaria* species. Ethanolic extracts of *Satureja khuzestanica* leaves exhibited antifungal activity against *Alternaria*, *Fusarium*, *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* species with MIC values ranging from 625-5000µg/ml [59]. In the present study, aqueous leaf and stem extracts of *M. charantia* exhibited 10mg/ml MIC against *A. alternata*. Similarly, Umedum (2013)[60] has reported MIC value of aqueous (100 mg/ml) and ethanolic (50mg/ml) extracts of *Eupatorium odoratum* leaf against *A. alternata*.

While comparing the efficacy of various plant part extracts of *M. charantia* in growth inhibition of *A. alternata* it was observed that their respective dilutions shows a strong dependence on extract concentrations as well as on solvent used for the extraction. In general, the antifungal activity of aqueous extract is less effective, whereas among the ethanolic extracts of experimental plant, the extract dilutions are the main factor in revealing the effectiveness of the antifungal activity as compared to crude extracts. These results revealed that the antifungal activity of the crude extracts was enhanced by increasing the concentration of the extracts; in fact, the inhibitory activity of the extracts was concentration dependent. This finding is in agreement with the report of Banso *et al*. (1999)[61], who also observed that higher concentrations of antimicrobial substances showed more growth inhibition. Similarly, in our case, when higher concentrations of the extracts were tested, the inhibition was higher, whereas the

lower concentration (diluted) inhibition was lower. This may be due to the concentrations of the phytoconstituents i.e. high in higher concentrations and low in lower concentration.

The antifungal activity of various plant parts of *M. charantia* can also be justified on the basis of the presence of good amount of various types of phtoconstituents like phenolic, tannin, flavonoids and saponin. The antifungal potential of phenolics and tannins from plant extracts has already been proved by various researches against the pathogenic fungal species [62-64]. Flavonoids and related polyphenols are reported to protect the plants against microbial invasion [65] and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microoraganisms. Saponins detected in various plant extracts have also shown antifungal activities[66-68]. Secondly most of the phytoconstituents are soluble in ethanol therefore the better extracting power of ethanol might be the other reason for the good antifungal activity of ethanolic extract of various plant parts of *M. charantia* as compared to water extract. It has been proved beyond the doubt that the effect of the extract on fungal inhibition depends upon the solvent used for the extraction[63]

CONCLUSION

Examination of various concentrations of *M. charantia* extracts on *A. alternata* in this study showed promising prospects for the utilization of natural plant part extracts. The ethanolic extracts of various part of *M. charantia* have great potential as antifungal compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases in plants caused by *A. alternata*. *In vitro* experiments showed that ethanolic extracts of *M. charantia* could reduce the mycelial growth of *A. alternata*. So extracts can be used as a potential source of sustainable eco-friendly botanical fungicides, after successful completion of wide range trials.

ACKNOWLEDGMENT

The authors are highly thankful to the Head, School of Studies in Botany, Jiwaji University Gwalior for providing necessary facility and support to carry out this research work.

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