In vitro efficacy of *Momordica charantia* extracts against phytopathogenic fungi, *Fusarium oxysporum*

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ABSTRACT

The study is aimed at evaluating the antifungal potential of plant parts of *Momordica charantia* against the *Fusarium oxysporum*. During investigation ethanolic extracts of stem showed maximum inhibition in both, spore germination (at 50mg/mL) and mycelial growth (at 50% concentration) with 86.10±4.80 and 79.04±1.06 % inhibition respectively. This was followed by root (with 64.81±3.20 and 62.78±2.85%), leaves (64.28±0.00 and 59.55±0.99%), and fruit (50.97±3.40 and 43.99±1.85%). Aqueous extracts of all plant parts showed a comparatively less significant amount of inhibition in spore germination and mycelial growth. Aqueous extracts (at 50 mg/mL concentration) of root showed 48.88±3.85% inhibition in spore germination followed by fruit (39.39±5.24), leaves (37.03±3.20%) and stem (33.32±3.04%). Even at 50% concentration, aqueous extracts of leaves (14.86±1.00%), root (13.11±1.23%), stem (7.04±0.98%), and fruit (4.05±2.01%) was not found effective in inhibiting the mycelial growth of *F. oxysporum*. Ethanolic extracts of fruit showed 0.625 mg/mL MIC value against *F. oxysporum* while ethanolic extracts of the leaves and root exhibited 2.5 mg/mL and stems 1.25 mg/mL MIC for *F. oxysporum*. The plant parts of *Momordica charantia* were also found rich in phenolics, tannins, flavonoids and saponins. These compounds may be responsible for the antifungal activity of respective plant part.

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INTRODUCTION

Fungal contamination of agricultural production is a chronic problem in developing countries and results in a decline in quality and quantity. According to an investigation, pathogenic fungi and various pests cause nearly 20% decrease in the yield of major food and crops (Agrios, 2000). Pathogenic fungi attack plants and cause diseases mainly in fresh fruits and vegetables in condition of high moisture contents and high temperature (Boyraz and Ozcan, 2006). *Fusarium* is considered as one of the most important groups of fungi, because of its diversity, cosmopolitan nature and the ability to cause serious diseases in plants. Vascular wilt, corm rot, root rot, and damping-off are some common diseases caused by the *Fusarium* (Smith *et al*., 1988).

Plant diseases are mostly controlled by chemical pesticides and in some cases by cultural practices. No doubt the use of chemicals has been found to be effective in controlling these diseases, but some major problems threaten to limit the continued use of these chemical fungicides. One problem is the tendency of fungi to develop resistance to chemicals, necessitating a higher dose or the development of new chemicals to replace those to which fungi are resistant (Bajwa *et al*., 2003). Another is the creation of a hazardous environment both for human beings and other flora and fauna by these chemical fungicides because of their non-biodegradable nature (Hayes and Laws, 1991). Furthermore, some synthetic pesticides are currently banned in several countries, and others are in continuous process. These problems highlight

the need to develop alternative methods for controlling plant diseases; this in turn has stimulated research on the occurrence of natural botanical extracts and the potential for commercialization of these materials (Arnason *et al*., 1989). Plant extract is recently advocated by several researches, as a potential control method of plant diseases (Belabid *et al*., 2010). Many plant extracts and essential oils have been reported to possess antimicrobial properties (Mathur and Gurjar, 2002; Tomar and Chandel, 2006; Pandey and Prasad, 2007; Sitara *et al*., 2008; Mesta *et al*., 2009; Gupta and Bhadauria, 2012; Gupta *et al*., 2014).

Momordica charantia Linn. commonly known as bitter gourd is tropical and subtropical climber of the family Cucurbitaceae. It is widely distributed in China, Malaysia, India and tropical Africa (Gupta *et al*., 2011). All parts of the plant, including the fruit taste very bitter, as it contains a bitter compound called momordicin. In Ayurveda, various parts of *M. charantia* are recommended for many diseases (Kirtikar and Basu, 1987). *M. charantia* contains an array of biologically active plant chemicals including triterpens, proteins, steroids, alkaloids, saponins, flavonoids and acids due to the presence of which the plant possesses anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic and anti-carcinogenic properties (Scartezzini and Speroni, 2000; Grover and Yadav, 2004; Beloin *et al*., 2005).

Plant active compounds are often rapidly degraded in soil, they generally have no mammalian toxicity, and they can have an effective role in sustainable agriculture (Saxena, 1983). Active compounds may play an important role in the defence mechanism of higher plants against various pathogens. Studies have already proved that active compound of the plant extracts may be responsible for their antifungal (Wang and Ng, 2002; Xia and Ng, 2004; Battinelli *et al*., 2006; Khanna and Kannabiran, 2008; Sharma and Kuma, 2009; Cheng *et al*., 2010; Akila *et al*., 2011; Glazer *et al*., 2012; Reddy *et al*., 2012), antibacterial, antimicrobial (Ordonez *et al*., 2006) and antioxidant (Veigas *et al*., 2007; Gordon *et al*., 2011) activities.

Therefore the objective of this study was to test the antifungal activity of aqueous and ethanolic extracts of leaf, fruit, stem, and root of *M. charantia* against *Fusarium oxysporum* (Nectriaceae) Schle. Simultaneously tested plant parts were subjected to phytochemical estimation to predict the relationship between quantity of some important secondary metabolites and their antifungal efficacy.

MATERIALS AND METHODS

Selection and collection of experimental materials

Leaves, fruits, stem and roots of *M. charantia* have been selected on the basis of their medicinal potential and uses in Ayurveda, Homeopathy and in traditional system of medicine as well as on the basis of their availability and abundance in the vicinity. Leaf, stem, and roots of *M. charantia* were collected from the plants grown in the botanical garden of the University, while the fruits were obtained from the local market.

Test organisms

Based upon the preliminary study of the pathogenic fungal species involved with the diseases of important agricultural crops, one important fungal species, *F. oxysporum* (ITCC # 6246) has been selected as test organisms. The test organism was obtained from the Indian Type Culture Collection Centre (ITCC), IARI, New Delhi. These fungal strains were maintained and sub-cultured on Potato Dextrose Agar at 26 ±1 °C and stored at 4^{o}C for further use.

Preparation of plant extracts

Aqueous extract of each sample was prepared by immersing 10 g dried powder in different conical flasks containing 40 mL of hot distilled water, stoppard with aluminium foil. The flasks were kept in a water bath for 20 minutes at 80-85 ^{o}C, and were allowed to cool and percolate for 24 hrs. Extracts were filtered using muslin cloths and the filtrate was centrifuged at 5000 rpm for 30 minutes. Obtained extracts were evaporated using a water bath and crude extracts were re-dissolved in 100 mL of distilled water, mixed

well, and filtered using Millipore filter (millipore 0.2 mm) to make it free from any type of contamination. All the extracts were directly assayed against the test organisms to determine the antifungal properties (Davis, 1956).

For the ethanolic extracts, the powdered sample of plant materials (10 g) was packed into a Soxhlet apparatus and extracted exhaustively with 100 mL of ethyl alcohol (80%) for 4 hrs at 60-80^{o}C temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman's filter paper (No. 1) and the ethanol was evaporated using a water bath. Finally ethanolic extract was prepared by re-dissolving crude extract in 100 mL of sterilized distilled water, mixed well, and filtered using a millipore filter. All the extracts were directly assayed against the test organisms to determine the antifungal properties (Davis, 1956).

Preparation of spore suspension

The spore suspensions of *F. oxysporum* were obtained from their respective 10-day old culture (grown on PDA media incubated at 25-30^{o}C). An agar disc of fungal inoculums (5 mm in diameter) was removed from seven - day old culture and was suspended in sterile distilled water to obtain a homogenous spore suspension of 1×10^{8} spores/mL. Spore count was determined and maintained using a counting chamber of haemocytometer. The spore suspension was immediately used for the spore germination assay (Bajpai *et al*., 2008)

Antifungal activity assay

The antifungal activity of aqueous and ethanolic extracts of leaf, fruits, stem, and roots of *M. charantia* were evaluated against plant pathogenic fungal species *F. oxysporum*. The antifungal activity of the plant parts extracts was evaluated by growth inhibition measurements and spore germination method. Minimum Inhibitory Concentration (MIC) of plant extracts was also calculated.

Growth inhibition measurements

In Poison Food Technique, diameter growth test was used to evaluate the toxicity of the extracts against the pathogen. Aqueous and ethanolic extracts of each part were prepared by re-dissolving crude extract separately in 100 mL of sterilized distilled water. Different concentrations (10, 20, 30, 40, and 50%) of aqueous and ethanolic extracts were subjected to antifungal activity assay. To get the required concentrations (10, 20, 30, 40, and 50%), plant part 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, 5 mm disc was removed using a cork borer from 7-day old culture of *F. oxysporum* and then transferred upside down into the center of each Petri plate. Five replicates were maintained for each concentration. Petri dishes of PDA medium without plant extracts served as control. The plates were incubated at 26±1 ºC for 8-days. The colony diameter was measured after the seven days of incubation period (Grower and Moore, 1962). After incubation the diameter of fungal colony was measured (in mm) and percentage inhibition of mycelial growth of test fungi was calculated (Deans and Svoboda, 1990).

Spore germination assay

Five concentrations of plant extracts along with control were separately tested for spore germination of *F. oxysporum*. The aqueous extract of each plant part was prepared by re-dissolving 100 mg crude extract separately in 1 mL of sterilized distilled water while for ethanolic extract, 100 mg crude plant extracts were re-dissolved in 1 mL of 10% DMSO. Conidial suspension of test organism was prepared in sterilized tap water and spore concentration was adjusted to 1×10^{8} spores/mL. Five concentrations of aqueous and ethanolic extracts (10, 20, 30, 40 and 50 mg/mL) and one control without plant extracts (10% DMSO with sterile distilled water for ethanolic extract) were separately tested for spore germination of *F. oxysporum*.

The conidial suspension was taken in different Eppendorf tubes and specific concentration of plant part extracts (aqueous and ethanolic) was mixed well in same eppendorfs tube. Controls without extracts were also maintained. The tubes were incubated at

26±1°C for 18 hrs. After 18 hrs the test solutions were placed in both the chambers of a haemocytometer by carefully touching the edges of the cover slip with the pipette tip and allowed to fill the counting chamber. Germination of spores was counted under a compound microscope by using haemocytometer cell counting method. All experiments were conducted in triplicate (Rana *et al*., 1997). The percent inhibition of spore germination was calculated (Mohana and Raveesha, 2007).

Minimum Inhibitory Concentration (MIC)

The MIC of the aqueous and ethanolic extracts of plant parts of *M. charantia* was determined by two fold dilution method against *F. oxysporum*. A stock solution of both the extracts was prepared by redissolving the 100 mg of the crude extract of each plant part in 1mL distilled water (for aqueous extract) and 1 mL of 10% DMSO for ethanolic extract. Experimental solutions of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39mg/mL were prepared by two fold dilutions from the stock solution. A 200 µL extract of each experimental solution was added separately to test tubes containing PDB (700 µL) and 100 µL spore suspension of the test fungi to make the final concentration of 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 mg/mL, respectively. The control tubes containing 700 µL PDA, 200 µL distilled water and 100 µL of spore suspension for aqueous extract and 700 µL PDA and 200 µL 10% DMSO and 100 µL of spore suspension for ethanolic extract were also maintained. These tubes were incubated for 2-7-days at 25±1°C. The lowest concentration of the plant extracts that prevented the fungal growth was used to determine the MIC, which were expressed in mg/mL (Murray *et al*., 1995).

Phytochemical analysis

The dried powder of each plant part of *M. charantia* was subjected to phytochemical analysis to quantify the alkaloids (Harborne, 1973), flavonoids (Zhuang *et al*., 1992), total phenolics and tannins (Makkar *et al*., 1993) and saponins (Obadoni and Ochuko, 2001).

Statistical analysis

RESULTS AND DISCUSSIONS

Antifungal activity assay

The results showed that growth increased with incubation time but mycelial growth was considerably reduced with increasing concentration of plant part extracts. Overall, results indicated that among both the tested extracts, ethanolic extracts of all parts of the *M. charantia* were most effective. Ethanolic extracts were found effective in inhibiting the mycelial growth of *F. oxysporum* at 50% concentration after 8th day (Table 1 and Fig.1). The test organism *F. oxysporum* was not found susceptible in aqueous extracts of leaves, stem, fruit, and root of *M. charantia*. None of the extracts was found effective in inhibiting the mycelial growth of *F. oxysporum* at all concentrations even after 8 days of incubation period (Table 1 and Fig.2). Among all plant parts, aqueous extracts of leaves exhibited more impact than by root, stem and fruit at 50% even after 8 days of incubation period (Table 1).

Spore Germination assay

As the extracts concentration increased, a reduction in the percentage of spore germination was observed. The spores of the control germinated after 16 h of incubation at 28°C in PDA medium. Ethanolic extracts of leaves, stem, fruits and root were found highly effective in inhibiting the spore germination of *F. oxysporum* at 50mg/mL. The most pronounced reduction in spore germination of *F. oxysporum* was recorded in the ethanolic extract of stem trailed by root, leaves and fruits at 50mg/mL (Table 2).

Minimum Inhibitory Concentration (MIC)

The lowest MIC was recorded in ethanolic extracts as compared to aqueous extracts. MIC value of 0.625 mg/mL was obtained in ethanolic extracts of fruit while ethanolic extracts of the leaves and the root exhibited the same 2.5 mg/mL and stems 1.25 mg/mL MIC. No MIC was recorded in aqueous extracts of fruit (>40) and root (>40) whereas the aqueous extracts of leaves and the stem showed 10 and 20 mg/mL MIC respectivel.

Table 1. Effect of aqueous and ethanolic extracts of leaves, stem, fruit, and seeds of *M. charantia* on the mycelial growth of *F. oxysporum* on 8th day.

Conc. (%)	Percent Inhibition (%)							
	Leaves		Stem		Fruit		Root	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
10	4.50 ±2.01^{c}	19.99 ±0.78^{e}	4.85 ±0.98^{b}	5.35 ±0.50^{a}	4.05 ±2.01^{a}	14.66 ±0.49^{e}	5.42 ±1.01^{c}	19.99 ±1.27^{e}
20	5.40 ±2.25^{c}	43.99 ±0.99^{d}	5.72 ±0.98^{ab}	7.58 ±1.22^{c}	4.05 ±2.01^{a}	19.55 ±0.61^{d}	6.77 ±1.01^{bc}	31.62 ±1.62^{d}
30	7.20 ±1.88^{bc}	52.88 ±0.60^{c}	6.16 ±1.20^{ab}	7.58 ±1.22^{c}	4.05 ±2.01^{a}	27.55 ±0.92^{c}	8.59 ±1.24^{b}	44.64 ±1.04^{c}
40	9.45 ±1.00^{b}	57.77 ±0.78^{b}	6.16 ±0.92^{ab}	14.28 ±0.49^{b}	4.05 ±2.01^{a}	35.55 ±1.57^{b}	11.30 ±1.01^{a}	51.62 ±1.94^{b}
50	14.86 ±1.00^{a}	59.55 ±0.99^{a}	7.04 ±0.98^{a}	20.97 ±1.22^{a}	4.05 ±2.01^{a}	43.99 ±1.85^{a}	13.11 ±1.23^{a}	62.78 ±2.85^{a}

Means in columns that do not share a superscript letter are significantly different 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 *F. oxysporum*.

Conc. (mg/ mL)	Percent Inhibition (%)							
	Leaves		Stem		Fruit		Root	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
10	18.51 ±3.70^{b}	18.75 ±0.00^{e}	19.69 ±5.24^{b}	27.44 ±3.40^{c}	18.66 ±4.61^{c}	28.20 ±2.21^{c}	13.33 ±2.88^{d}	18.66 ±2.30^{c}
20	27.77 ±2.40^{ab}	29.16 ±3.60^{d}	28.78 ±2.62^{ab}	29.40 ±5.88^{c}	22.22 ±2.40^{bc}	36.0 ±4.00^{bc}	16.66 ±0.00^{cd}	21.33 ±2.30^{c}
30	30.00 ±0.00^{a}	37.77 ±3.85^{c}	30.15 ±2.74^{a}	35.55 ±3.85^{c}	29.62 ±3.21^{ab}	41.26 ±5.49^{ab}	20.36 ±3.21^{c}	26.08 ±4.35^{c}
40	33.32 ±5.55^{a}	51.11 ±3.85^{b}	31.66 ±2.88^{a}	66.66 ±4.12^{b}	31.47 ±3.21^{ab}	45.60 ±3.03^{ab}	29.41 ±0.00^{b}	46.96 ±2.62^{b}
50	37.03 ±3.20^{a}	64.28 ±0.00^{a}	33.32 ±3.04^{a}	86.10 ±4.80^{a}	39.39 ±5.24^{a}	50.97 ±3.40^{a}	48.88 ±3.85^{a}	64.81 ±3.20^{a}

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).

Phytochemical Analysis

All the dried and powdered samples of plant parts of *M. charantia* were analyzed for the quantitative estimation of phytoconstituents. The quantitative estimation of the phytochemicals has shown that fruit (on dry matter basis) contained higher amount of total phenolics and tannins and least amount of flavonoids and saponin. Leaves were found to have the highest amount of saponin than by total phenolics and flavonoids while tannin was not detected in leaf. Stem was found to contain total phenolics, tannins, saponins, and flavonoids. Root was found to have total phenolics and saponins. Flavonoids and tannins were not detected in roots of *M. charantia* (Table 3). Since the use of natural products for the control of fungal diseases in plants is considered as an appropriate alternative to synthetic fungicides due to their

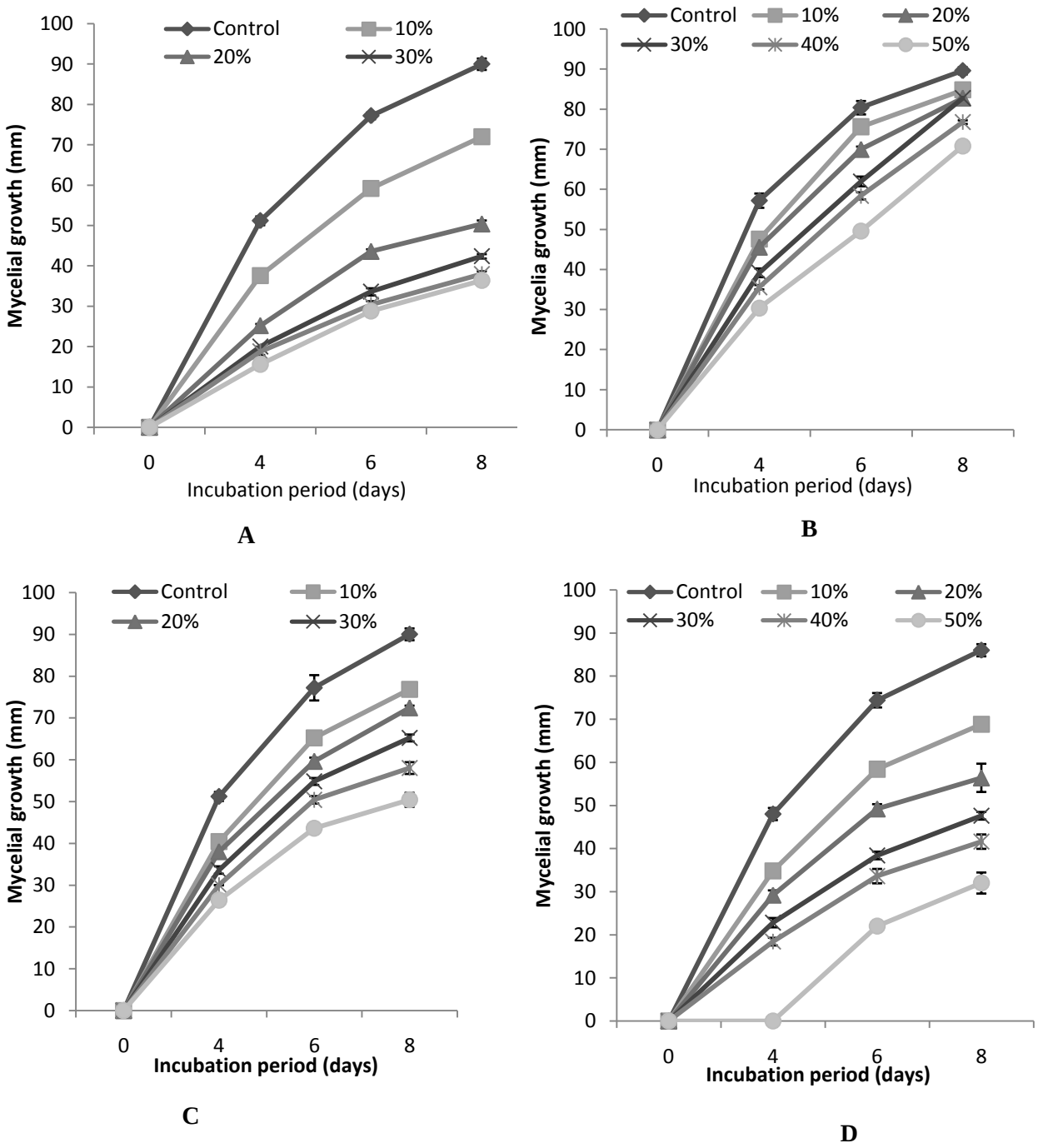

Fig. 1. Mycelial growth diameter (mm) of *F. oxysporum* at various concentrations of ethanolic extracts of leaves **(A)**, stem **(B)**, fruit **(C)** and root **(D)** of *M. charantia*.

safe impact on the environment (Cao and Forrer, 2001). In recent years, consumer demand for effective, safe natural products to control food spoilage without chemical residues has increased. Plant extracts, essential oils and aromatic volatile products of plant secondary metabolism, have formed the basis of many applications in food flavoring and preservation industries (Rahman and Kang, 2009; Tsigarida *et al*., 2009). Among all the screened plant parts of *M. charantia*, the aqueous and ethanolic

Table 3. Quantitative estimation of phytoconstituents of *M. charantia*

Phytocons tituents (%)	Quantity of Phytoconstituents (g/100g)			
	Leaves	Stem	Fruit	Root
Phenolics	0.880 ±0.06	3.200 ±0.18	4.280 ±0.56	0.500 ±0.07
Tannins	ND	2.70 ±0.17	3.40 ±0.65	ND
Flavonoids	0.632 ±0.10	0.216 ±0.02	0.131 ±0.01	ND
Saponin	2.050 ±0.01	1.350 ±0.2	1.220 ±0.03	1.250 ±0.03

ND – Not detected

extracts of leaf exhibited moderate and strong antifungal activity against the tested fungi. This is in agreement with the findings of Leelaprakash *et al*. (2011), who also made similar observations and reported that aqueous leaf extracts also have antimicrobial activity. Ethanolic leaf extract and essential oil of *M. charantia* seeds have also been reported to possess strong antimicrobial activity against the bacterial strain of *Staphylococcus aureus* (Braca *et al*., 2008; Coutinho *et al*., 2010). Mwambete (2009) and Jagessar *et al*. (2010) 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 Shinde and Dhale (2011) with leaf extracts from *Ocimum tenuiflorum* and *Datura stramonium* against *Fusarium oxysporum* and *Rhizopus stolonifer*; Jalander and Gachande (2012) with plant extracts from *Tinospora cordifolia* against *Fusarium oxysporum* and *Alternaria solani*.

Burger *et al*. (2010) also reported better effect of ethanolic extracts of the leaf of *Momordica* species on spore germination of *F. oxysporum* and *A. solani* than the aqueous fractions of leaves. In contrast to this, Sharma and Trivedi, (2002) and Gupta and Tripathi, (2011) have reported good antifungal activity in aqueous leaf fractions of the *Datura stramonium* ,*Calotropis procera* and *Solanum torvum* against the *F. oxysporum* and *F. sacchari*. This could be attributed to the fact that antifungal compounds present in the leaf extracts might have extracted better in these organic solvents than aqueous extract (Kagale *et al*., 2004).

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 contain higher concentrations of active antimicrobial agents than aqueous extracts and therefore show higher fungitoxic activity than aqueous fractions. During the investigation, aqueous extracts of the stem of *M. charantia* significantly inhibited the mycelial growth of *F. oxysporum*. Antifungal potential of aqueous stem extracts of *Cymbopogon proximus* and *Zingiber officinale* against the growth of *F. oxysporum* and *A. alternata* has already been reported by Fawzi *et al*. (2009). In the present investigation, ethanolic extracts of the stem of *M. charantia* also exhibited better fungitoxic activity against the tested fungal species including strong inhibition of spore germination of *F. oxysporum.* Effectiveness of ethanolic stem extracts of *Ruta graveolens* against the growth of *F. oxysporum* has been reported by Pandey *et al*. (2011). The good fungitoxic activity of the stem extracts (aqueous and ethanolic) of *M. charantia* against the mycelial growth as well as spore germination of *F. oxysporum* has been reported. Reports are also available on the inhibition of spore germination of *F. oxysporum* by stem extracts of *Capparis deciduas*, *Lantana camara* and *Tridax procumbens* (Sharma and Kumar, 2009). 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 positive for their role in plant defense mechanism (Okwu, 2004). In the present study aqueous extracts of fruit was found less effective against the mycelial growth of *F. oxysporum* whereas ethanolic fruit extracts showed good antifungal activity on the spore germination as well as mycelial growth of *F. oxysporum.* This is in agreement with the earlier findings of Parihar and Kumar (2013); Gupta and Bhadauria (2012), and (Gupta *et al*., 2014). Effectiveness of ethanolic fruit extracts may be due to the presence of active compounds. Gupta and Banarjee (1972) observed strong inhibitory effect of various species of *Curcuma* and *Brassica* against *Aspergillus niger* and *Trichophyton rubrum.* Stange *et al*. (1999) isolated a phytoalexins from the fruit tissue of *Cucurbita maxima* and reported it as an induced antifungal compound. Indeed, plants of family cucurbitaceae, including *M. charantia*, produce a number of proteins and peptides that have antifungal potential. It includes trypsin inhibitors, lectins (Wang and Ng, 1998), ribosome-inactivating proteins (Gao *et al*., 1994; Prakash *et al*., 2002; Wang and Ng, 2003; Xu *et al*., 2007) and ribonucleases (Fong *et al*., 2000). In this study ethanolic root extracts of *M. chrantia* was found most effective against the mycelial growth and spore germination of *F. oxysporum* as observed previously (Balkhande and Surwase, 2013; Bembrekar and Ingle, 2013).

Leaves, stem, fruits and root extracts of *Momordica charantia* plant extracts against *F. oxysporum* showed variation in their MIC values. However, MIC of ethanolic fruit extracts of *M. charantia* was 0.625mg/mL for *F. oxysporum*. Inhibitory activity of *M. charantia* seed essential oil was evidenced against *Candida albicans* with MIC value >500µg/mL (Braca *et al*., 2008). Antibacterial activity of the seed extracts of *M. charantia* with MIC 300-500mg/mL (Roopashree *et al*., 2008) and 100g/mL MIC against *Alternaria solani* by ethanolic seed extracts of *Voacanga africana* have been reported by Duru and Onyedineke (2010). Present investigation reveals that ethanolic leaf extracts of *M. charantia* showed MIC values against *F. oxysporum.* The best antifungal activity of leaf extracts of *Bucida buceros* against *F. oxysporum* has been reported earlier (Sadeghi-Nejad *et al*. 2010, Bajpai and Kang, 2012). In the present study, aqueous leaf extracts exhibited 20mg/mL MIC against *F. oxysporum*. Lee *et al*. (2007) have reported 5mg/mL and 13.3mg/mL MIC value of hot water and methanolic extracts of *Cinnamomum cassia* against *F. moniliforme* respectively. While 0.5mg/mL MIC of the crude leaf extracts of *Solanum torvum* against *F. sacchari* was observed by Gupta and Tripathi (2011). The antifungal activity of plant parts of *M. charantia* can also be justified on the basis of the presence of s substantial quantity 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 (Duk, 2002: Castillo *et al*., 2010; Zuniga *et al*., 2012). Multiple biological activities of flavonoids such as antibacterial, antifungal, and antiviral activities have already been reported. Flavonoids and related polyphenols are reported to protect the plants against microbial invasion (Harborne and Williams, 2000) 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

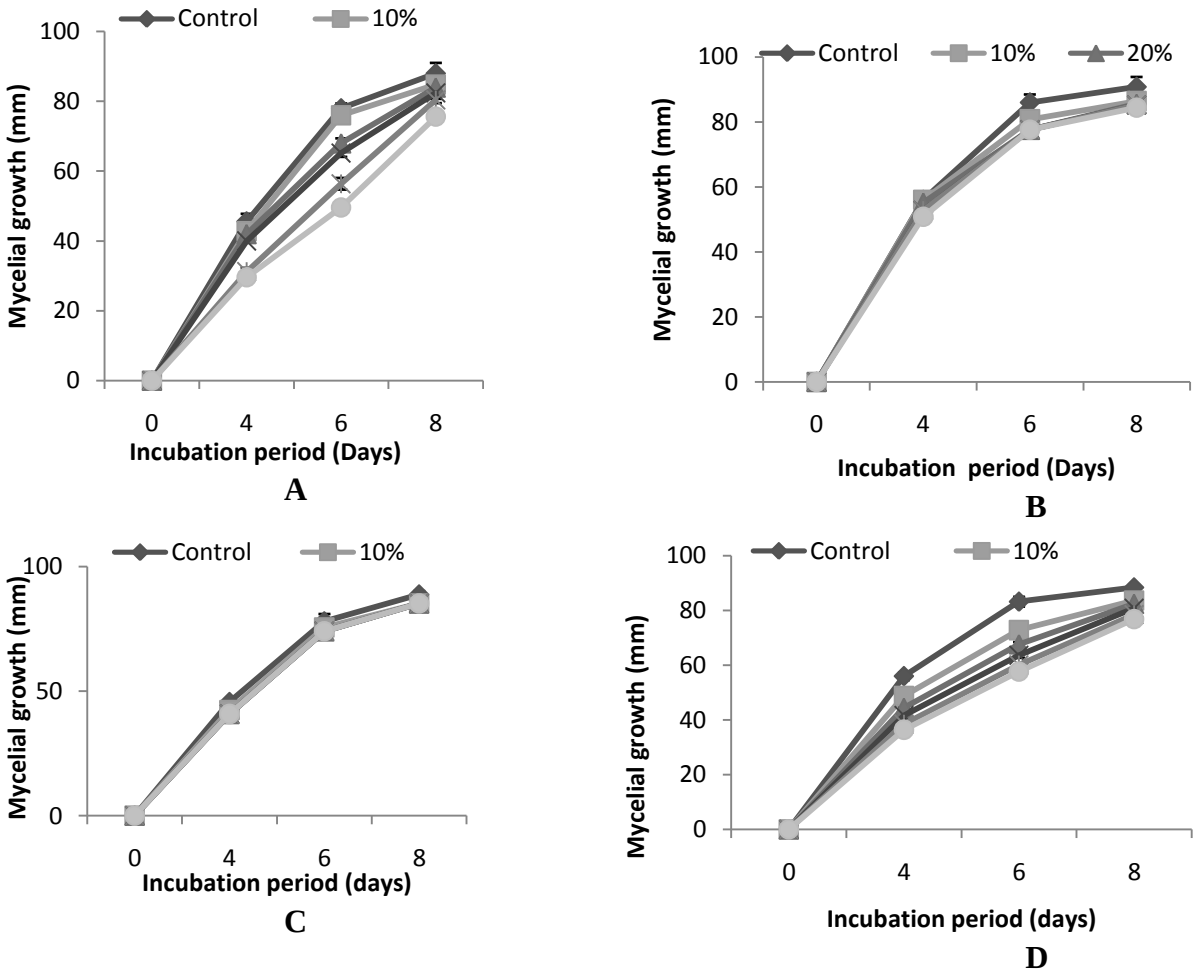

Fig. 2. Mycelial growth diameter (mm) of *F. oxysporum* at various concentrations of aqueous extracts of leaves **(A)**, stem **(B)**, fruit **(C)** and root **(D)** of *M. charantia*.

(Escalante *et al*., 2002; Iorizzi *et al*., 2002; Okwu, 2003). 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 plant parts of *S. cumini* as compared to water extract. It has been proved beyond doubt that the effect of the extract on fungal inhibition depends upon the solvent used for the extraction (Castillo *et al*., 2010).

The findings of this study showed promising prospects for the utilization of ethanolic extracts of leaves, fruit, stem, and root of *M. charantia* as a natural fungicide to manage plant pathogenic fungi *Fusarium oxysporum*. Results of the studies indicate that concentration of effective bioactive compounds in ethanolic extracts was higher than those in the aqueous extracts. Phytochemical studies also showed the presence of total phenolics, tannins, flavonoids and saponins in *M. charantia* plant part extracts, which may be responsible for their therapeutic values and antifungal potential. Mycelial growth increased with incubation time but mycelial growth was considerably reduced with increasing concentration of plant part extracts. None of the aqueous extracts of *M. charantia* was found effective in inhibiting the mycelial growth of *F. oxysporum* at all concentrations even after 8 days of incubation period. Ethanolic extracts of various parts of *M. charantia* showed good antifungal potential against *F. oxysporum* in comparison to aqueous extracts. Ethanolic stem extracts of *M. charantia* exhibited strong inhibition against the spore germination of *F. oxysporum*. The prospect of using this plant

part extracts for development of natural fungicides is appealing and acceptable. This plant and its parts are readily available in normal conditions. Moreover, it can also prove to be a helpful resource for economically weaker farmers.

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