

Full Length Research Paper

Antibacterial, antifungal and phytotoxic activities of *Luffa cylindrica* and *Momordica charantia*

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Plants are important for their medicinal value because they contain numerous compounds with therapeutic value. Keeping in mind this aspect, traditionally important plants, *Luffa cylindrica* and *Momordica charantia*, were screened for antibacterial, antifungal and phytotoxic activities. The *n*-hexane fraction of *L. cylindrica* exhibited good (64%) and crude methanolic extract, moderate (58%) antibacterial activity against *Bacillus subtilis*. Butanol (BuOH) fraction presented moderate activity (58%) against *S. flexenari*. The crude methanolic extract and chloroform fraction (CHCl₃) of *M. charantia* showed good (60 and 64%) antibacterial activity against *Escherichia coli*, *B. subtilis*, respectively. The BuOH fraction of *L. cylindrica* showed significant antifungal activity against *Fusarium solani* (85%) and *Trichophyton longifusus* (80%). The crude methanolic extract and ethyl acetate fraction (EtOAc) presented good linear growth inhibition against *Microsporum canis* (70%). The crude methanolic extract of *M. charantia* showed good antifungal activity against *Aspergillus flavus* (70%), *T. longifusus* (70%). The *n*-hexane fraction displayed excellent linear growth inhibition against *T. longifusus* (85%), EtOAc fraction against *A. flavus* (80%) and BuOH fraction against *Candida glabrata* (80%). The EtOAc fraction of *L. cylindrica* displayed moderate growth regulation of 41.66% against *Lemna minor* L at 1000 µg/ml, whereas at 100 and 10 µg/ml the aqueous fraction displayed growth promoting activity of 5.55 and 16.66%, respectively. The crude methanolic extract and CHCl₃ fraction of *M. charantia* displayed low growth regulation of 22.22% at 1000 µg/ml, whereas at 100 and 10 µg/ml the aqueous fraction displayed growth promoting activity of 11.11 and 27.77%, respectively.

Key words: *Luffa cylindrica* and *Momordica charantia*, antibacterial, antifungal, phytotoxic.

INTRODUCTION

According to a survey about 8000 plants have medicinal value out of which 2000 species are found in Pakistan (Oliyiwola, 1984). Due to poor economic condition, most of the South Asian cannot afford the cost of the primary health care and thus depends on traditional medicinal plants for their therapeutic antenatal and postnatal cases (Aslam, 2002). In the global pharmaceutical market, 30% of the raw material is from medicinal plants. It is therefore important to investigate the medicinal flora of the country which will be helpful for the pharmaceutical sector of the country (Shinwari et al., 2003). After literature review, we

selected *Luffa cylindrica* and *Momordica charantia* of the family Cucurbitaceae.

L. cylindrica, locally called as 'Thoray' is famous for its fruit, eaten as vegetable and is good for health (Kirtikar and Basu, 1987). It has been used for the treatment of cynocytous, flu and other diseases in Asia. It is used as an anthelmintic, stomachic and antipyretic phytomedicinal drug (Kou et al., 2001). The fibers of *L. cylindrica* are mainly composed of cellulose (54.2%) and lignin (15.10%) and have been evaluated for the use in strengthening resin matrix compound materials (Kaufman et al., 1999).

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Seeds are famous for their emetic and cathartic properties. Young fruits have demulcent and appetizers properties and are exertive of mind, bile and phlegm. Fruits are used as vegetable in early stages whereas its medicinal uses increases with its maturity (Vashista, 1974). *M. charantia* locally called as 'Karela' is famous for its fruit and eaten as vegetable. The fruits are considered as tonic, stomachic, carminative agents and have been used for diabetes. The fruits are also used in rheumatism, gout and diseases of liver and spleen and are febrifuge. The seeds and leaves are used as anthelmintic. The leaves are also reported to be useful in piles, leprosy and jaundice (Vashista, 1974).

The leaves, fruits and roots have been used as laxative and antipyretic agents (Nayar and Singh, 1998). It has been used to treat anemia, cholera, bronchitis and ulcers. The fruit juice is used for the treatment of colic, wounds, sores and worms. The seeds have been reported to have antiviral, antiulcer and anti-leukemia properties (Sastri, 1962). MAP 30, a protein from *M. charantia*, has been reported to have anti-HIV and antitumor properties (Lee-Hung et al., 1995). Keeping in view the medicinal importance of *L. cylindrica* and *M. charantia*, the current study was carried out to find the antibacterial, antifungal and phytotoxic activities of these two plants.

MATERIALS AND METHODS

Plant material

The ripe fruits of *L. cylindrica* and *M. charantia* were collected from different parts of District Peshawar, Khyber Pakhtunkhwa, Pakistan.

Extraction

The collected fruits were cleaned, washed and kept in shade for drying. After drying they were chopped into small pieces and grounded to powder with an electric grinder. In methanol the powdered fruit of *L. cylindrica* (1 kg) and *M. charantia* (1 kg) were soaked for 3 days, twice, at room temperature, with occasional shaking. All the filtrates were concentrated, at 40°C, under vacuum, by rotary evaporator. Crude methanolic extract of *L. cylindrica* (269 g) and *M. charantia* (259 g) were obtained.

Fractionation

The crude methanolic extract of *L. cylindrica* (269 g) and *M. charantia* (259 g) were suspended in distilled water (500 ml) and partitioned with *n*-hexane (3×500 ml), CHCl₃ (3×500 ml), EtOAc (3×500 ml) and BuOH (3×500 ml) respectively to yield the *n*-hexane (24 and 28 g), CHCl₃ (48 and 35 g), EtOAc (35 and 42 g), BuOH (42 and 29 g) and aqueous (61 and 65 g) fractions, respectively. 60 g of *L. cylindrica* and *M. charantia* were left for biological/pharmacological activities.

Antibacterial activity

The crude methanolic extract and various fractions of *L. cylindrica* and *M. charantia* were screened for possible antibacterial activity

against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella typhi* employing agar well diffusion method (Bashir et al., 2011). On the sterile nutrient agar plates, 18 h old cultures from nutrient broth was transferred and spread to make bacterial lawn. Wells were made in the plates using a sterile 6 mm borer. The test samples were prepared by dissolving 3 mg of the test samples in 1 ml of DMSO, serving as stock solution. From each stock solution 100 µl was introduced to respective well and incubated for 24 h at 37°C. Amoxicillin was used as positive and DMSO as negative control. Percent zone of inhibition was measured in comparison with positive control.

Antifungal activity

The crude methanolic extract and various fractions of *L. cylindrica* and *M. charantia* were screened for antifungal activity against *Aspergillus flavus*, *Trichophyton longifusus*, *Candida albicans*, *Microsporium canis*, *Fusarium solani* and *Candida glabrata* following (Bashir et al., 2011). In sterile DMSO, stock solution was prepared by dissolving 24 mg/ml. Sabouraud dextrose agar (SDA) was prepared and incubated for 24 h at 28±1°C for sterility check. After incubation the sterile plates were used to refresh the aforementioned fungal species followed by incubation for seven days at 28±1°C. 4 ml of SDA was poured in test tubes for making slants followed by autoclaving. After autoclaving when the temperature of the media is about 50°C, 66.6 µl from the stock solutions of test samples were added into respective test tube and kept in slanted position. The tubes were incubated at 28±1°C for 24 h to check sterility. Seven days old fungal culture was then introduced into the labeled test tubes and incubated for seven days at 28±1°C. Miconazole and DMSO served as positive and negative controls. After seven days results were taken by measuring the linear growth on the slanted test tubes in comparison with negative control.

Phytotoxic bioassay

Phytotoxic activity of the crude methanolic extract and various fractions of *L. cylindrica* and *M. charantia* were determined against *L. minor* as per our reported procedure (Bashir et al., 2010). The stock solutions of the test samples were prepared in methanol (20 mg/ml). From the stock solutions, 1000, 100 and 10 µg/ml were transferred to sterilized conical flasks and left overnight for evaporation of methanol. 20 ml of E-media were then added to the conical flasks and 16 *L. minor* plants with a rosette of three fronds, were introduced into each flask. Paraquat and methanol served as positive and negative control respectively. All the flasks were then incubated in growth chamber at 28±1°C for 7 days. The results were determined by counting the number of plants damaged and growth inhibition was calculated in reference with the negative control.

RESULTS AND DISCUSSION

Antibacterial activity of *Luffa cylindrica*

The results of antibacterial activity of *L. cylindrica* are presented in Figure 1. The crude methanolic extract showed moderate activity against *B. subtilis* (58%) and *K. pneumoniae* (56%) while low activity was observed against *E. coli* (27%), *S. flexneri* (33%). The crude extract was inactive against *S. aureus* and *S. typhi*. The *n*-hexane

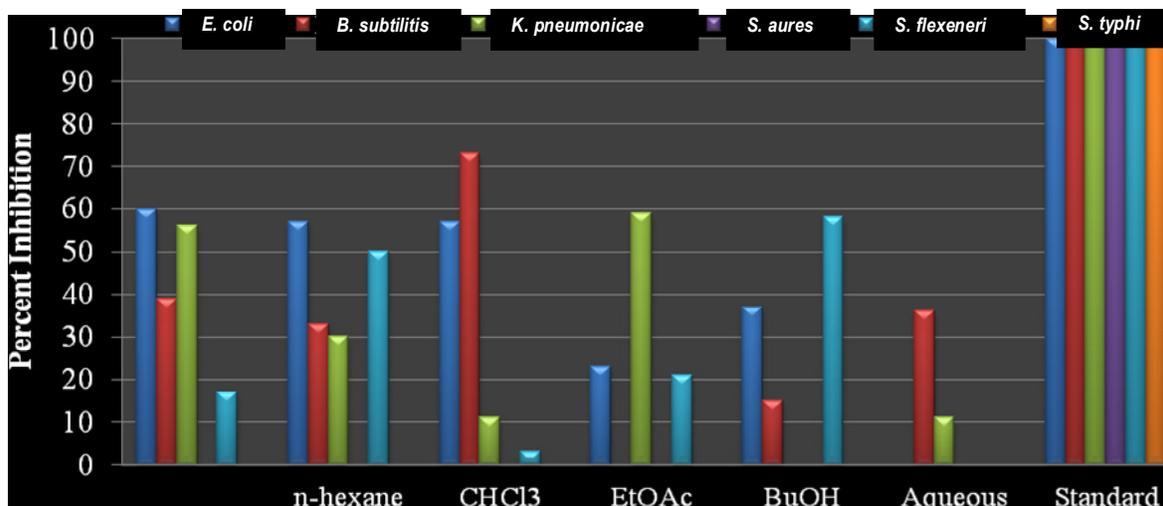


Figure 1. Antibacterial activity of crude methanolic extract and various fractions of *L. cylindrica*.

fraction exhibited good activity against *B. subtilis* (64%), low against *E. coli* (37%), *K. pneumonia* (30%) and *S. flexneri* (17%) while it was inactive against *S. aureus* and *S. typhi*. The CHCl₃ fraction presented moderate activity against *E. coli* (57%), low activity against *B. subtilis* (39%), *K. pneumonia* (11%) and *S. flexneri* (13%). It was inactive against *S. aureus* and *S. typhi*. The EtOAc fraction was moderately active against *K. pneumonia* (57%). It showed low activity against *E. coli* (23%) and *S. flexneri* (21%) while it was inactive against *B. subtilis*, *S. aureus* and *S. typhi*. BuOH fraction presented moderate activity against *S. flexneri* (58%), low activity against *E. coli* (37%) and *B. subtilis* (15%) while inactive against *K. pneumonia*, *S. aureus* and *S. typhi*. The aqueous fraction exhibited low activity against *B. subtilis* (36%) and *K. pneumonia* (11%) while it was inactive against *E. coli*, *S. flexneri*, *S. aureus* and *S. typhi*. Previously, we determined the antibacterial activity of crude methanolic extract and various fractions of *Zizyphus jujuba* against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. typhi*, *Bacillus pumilus*, *K. pneumoniae*, *S. pneumoniae* and *E. aerogenes*. Against *B. pumilus* and *P. aeruginosa*, the *n*-hexane and aqueous fractions showed activity of 60 and 66.66% respectively. Against *S. epidermidis*, *S. typhi*, *P. aeruginosa* and *B. pumilus*, EtOAc fraction showed 65.38, 62.96, 62.96 and 72% antibacterial activity respectively (Bashir et al., 2010). This time we also got some good results against various bacterial pathogens as stated.

Antibacterial activity of *Momordica charantia*

The results of antibacterial activity of *M. charantia* are presented in Figure 2. The crude methanolic extract of the plant showed good activity (60%) against *E. coli*, moderate (56%) against *K. pneumonia* and low activity

(39 and 17%) against *B. subtilis* and *S. flexneri*. The crude extract was inactive against *S. aureus* and *S. typhi*. The *n*-hexane fraction of the plant exhibited moderate activity against *E. coli* (57%) and *S. flexneri* (50%), low against *B. subtilis* (33%) and *K. pneumonia* (30%) while it was inactive against *S. aureus* and *S. typhi*. The CHCl₃ fraction showed good activity (64%) against *B. subtilis*, moderate (57%) against *E. coli* and low activity against *K. pneumonia* (11%) and *S. flexneri* (13%). It was inactive against *S. aureus* and *S. typhi*. The EtOAc fraction was moderately active (59%) against *K. pneumonia*. It showed low activity against *E. coli* (23%) and *S. flexneri* (21%) while inactive against *B. subtilis*, *S. aureus* and *S. typhi*. BuOH fraction was found moderately active against *S. flexneri* (58%), low active against *E. coli* (37%) and *B. subtilis* (15%) while inactive against *K. pneumonia*, *S. aureus* and *S. typhi*. The aqueous fraction exhibited low activity against *B. subtilis* (36%) and *K. pneumonia* (11%) while found inactive against *E. coli*, *S. flexneri*, *S. aureus* and *S. typhi*.

Antifungal activity of *L. cylindrica*

The results of antifungal activity of *L. cylindrica* are presented in Figure 3. The results showed that the crude methanolic extract presented good linear growth inhibition against *M. canis* (70%) and *T. longifusus* (60%), low against *F. solani* (35%), *C. albicans* (10%) and *A. flavus* (05%) and was found inactive against *C. glabrata*. The *n*-hexane fraction presented good linear growth inhibition against *C. albicans* (70%) and *M. canis* (70%), moderate against *T. longifusus* (60%) and low against *F. solani* (15%). The *n*-hexane fraction was inactive against *A. flavus* and *C. glabrata*. The CHCl₃ fraction showed good activity against *M. canis* (62%), moderate against *T. longifusus* (50%), low against *F. solani* (20%) and was

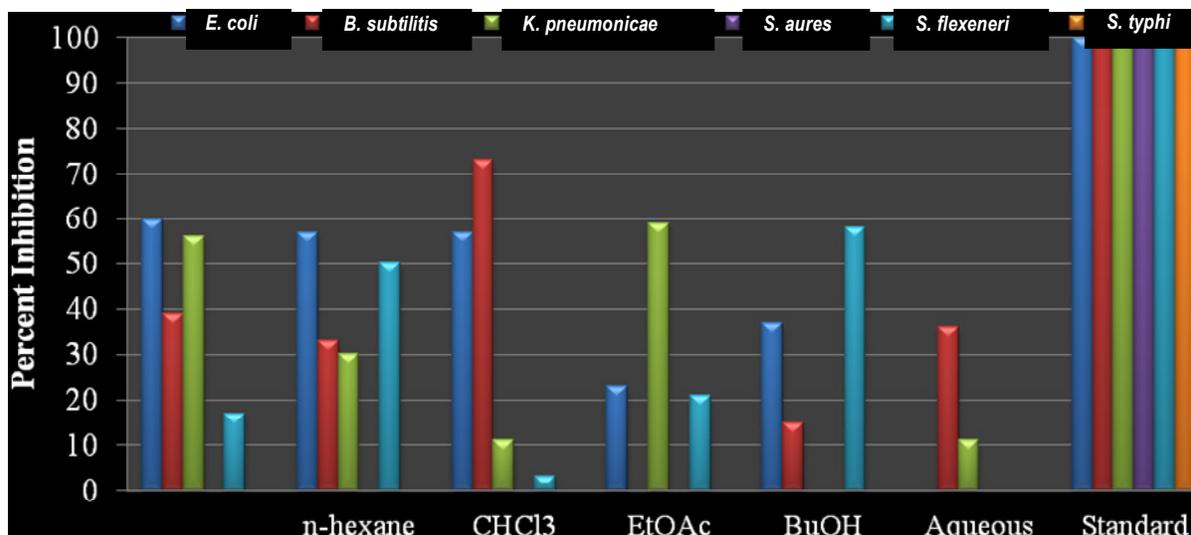


Figure 2. Antibacterial activity of crude methanolic extract and various fractions of *M. charantia*.

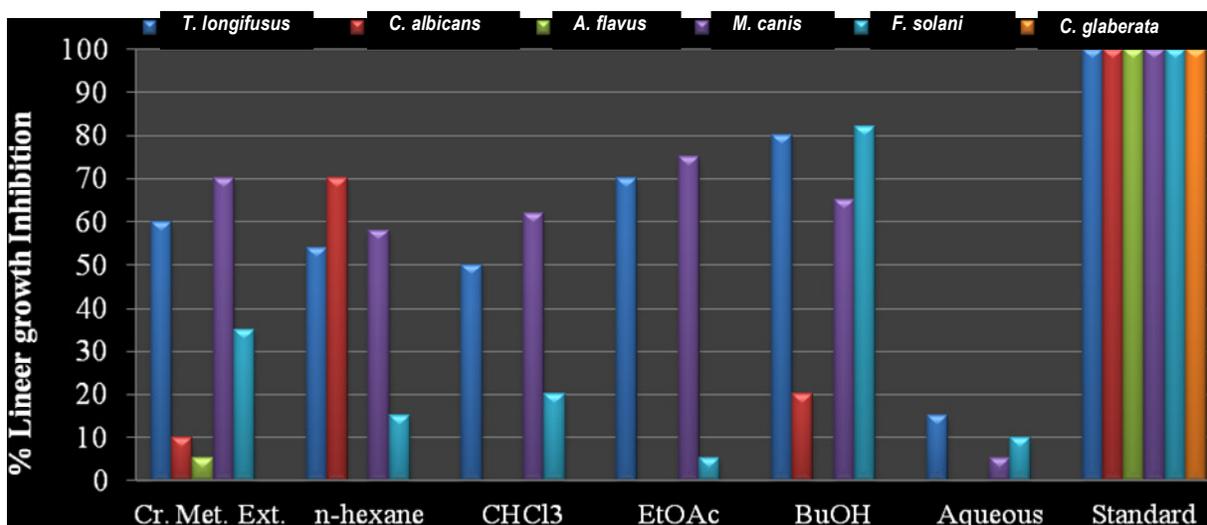


Figure 3. Antifungal activity of crude methanolic extract and various fractions of *L. cylindrica*.

found inactive against *C. albicans*, *A. flavus* and *C. glaberata*. The EtOAc fraction presented good linear growth inhibition against *M. canis* (75%) and *T. longifusus* (70%), low against *F. solani* (05%) and was found inactive against *C. albicans*, *A. flavus* and *C. glaberata*. The BuOH fraction showed significant inhibitory activity against *F. solani* (85%) and *T. longifusus* (80%), good against *M. canis* (65%) and low against *C. albicans* (10%). The fraction was found inactive against *A. flavus* and *C. glaberata*. The aqueous fraction presented low linear growth inhibition against *T. longifusus* (15%), *F. solani* (10%) and *M. canis* (05%) and inactive against *C. albicans*, *A. flavus* and *C. glaberata*. This work is in continuation of our previous work in which we determined the antifungal activity of crude methanolic extract and

various fractions of *Onosma griffithii* Vatke against *A. flavus* and *F. solani*. Against *A. flavus*, the crude methanolic extract and CHCl₃ fraction displayed moderate antifungal activity of 55 and 59% respectively while good and moderate activity was also observed against *F. solani* (40 and 60%, respectively) (Ahmed et al., 2009). This time we got some significant antifungal activities in some cases.

Antifungal activity of *Momordica charantia*

The results of antifungal activity of *M. charantia* are presented in Figure 4. The crude methanolic extract presented good linear growth inhibition against *A. flavus*

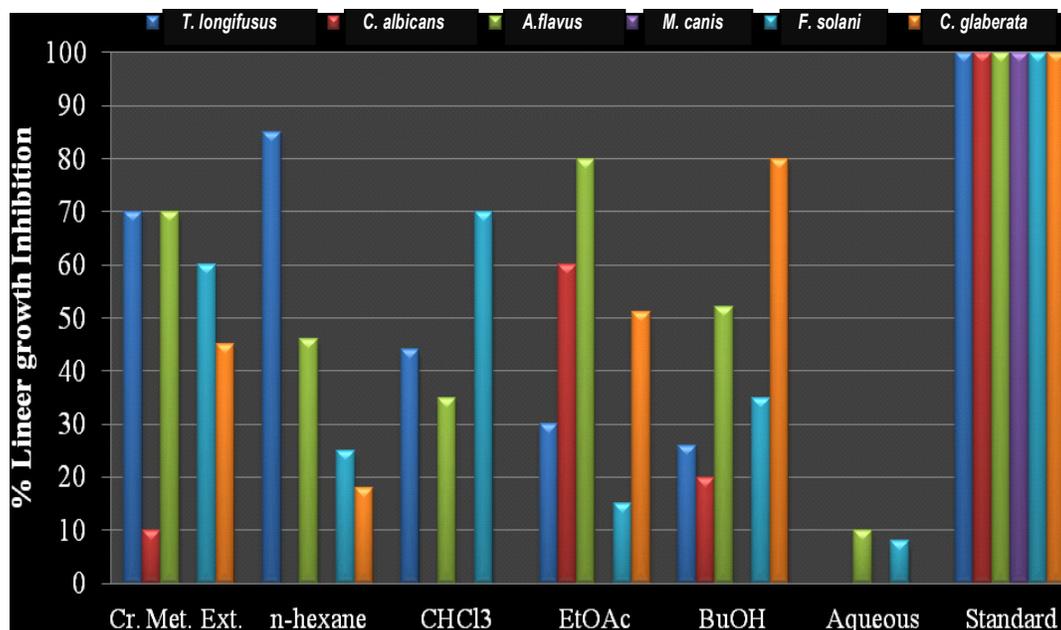


Figure 4. Antifungal activity of crude methanolic extract and various fractions of *M. charantia*.

(70%), *T. longifusus* (70%) and *F. solani* (60%), moderate against *C. glaberata* (45%) and low against *C. albicans* (10%). It was inactive against *M. canis*. The *n*-hexane fraction displayed excellent linear growth inhibition against *T. longifusus* (85%), moderate against *A. flavus* (46%) and low against *F. solani* (25%) and *C. glaberata* (18%). It was inactive against *C. albicans* and *M. canis*. The chloroform fraction displayed good linear growth inhibition against *F. solani* (70%), moderate against *T. longifusus* (44%) and low against *A. flavus* (35%). It was inactive against *C. albicans*, *M. canis* and *C. glaberata*. The ethyl acetate fraction presented excellent linear growth inhibition against *A. flavus* (80%), good against *C. albicans* (60%) and moderate against *C. glaberata* (51%). It showed low activity against *T. longifusus* (30%) and *F. solani* (15%) and inactive against *M. canis*. The Butanol fraction presented excellent linear growth inhibition against *C. glaberata* (80%), moderate against *A. flavus* (52%) and low against *F. solani* (35%), *T. longifusus* (26%) and *C. albicans* (20%). It was inactive against *M. canis*. The aqueous fraction presented low linear growth inhibition against *A. flavus* (10%) and *F. solani* (8%). It was inactive against *T. longifusus*, *M. canis*, *C. albicans* and *C. glaberata*.

Phytotoxic activity of *Luffa cylindrica*

The results of antifungal activity of *L. cylindrica* are presented in Figure 5. The crude methanolic extract displayed low growth regulation of 33.33, 19.44 and 2.77% at concentration of 1000, 100 and 10 $\mu\text{g/ml}$, respectively. The *n*-hexane fraction presented low growth

regulation of 25 and 5.5 at 1000 and 100 $\mu\text{g/ml}$ respectively. It showed no growth regulation at 10 $\mu\text{g/ml}$. The CHCl_3 fraction presented low growth regulation of 19.44 and 2.77% at 1000 and 100 $\mu\text{g/ml}$, respectively. It showed no growth regulation at 10 $\mu\text{g/ml}$. The ethyl acetate fraction displayed moderate growth regulation of 41.66% at 1000 $\mu\text{g/ml}$ and low regulation of 13.88 and 2.77% at 100 and 10 $\mu\text{g/ml}$, respectively. The BuOH fraction presented low activity at 1000 and 100 $\mu\text{g/ml}$ (30.55 and 11.11%). It was inactive at 10 $\mu\text{g/ml}$. The aqueous fraction presented no growth inhibition at 1000 $\mu\text{g/ml}$, whereas at 100 and 10 $\mu\text{g/ml}$ displayed growth promoting activity of 5.55 and 16.66%, respectively. Previously we determined the phytotoxic activity of *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* (Family Polygonaceae) against *Lemna minor* L at 1000, 100 and 10 $\mu\text{g/ml}$. Except *R. hastatus*, all the plants showed significant activity at a concentration of 1000 $\mu\text{g/ml}$. Moderate activity was shown by *R. australe*, *R. nepalensis* and *P. persicaria* at the concentration of 100 $\mu\text{g/ml}$ (Farrukh et al., 2010).

Phytotoxic activity of *Momordica charantia*

The results of antifungal activity of *M. charantia* are presented in Figure 6. The crude methanolic extract displayed low growth regulation of 22.22 and 0.5% at concentration of 1000 and 100 $\mu\text{g/ml}$, respectively and inactive at 10 $\mu\text{g/ml}$. The *n*-hexane fraction presented low growth regulation of 19.44 and 11.11% at 1000 and 100 $\mu\text{g/ml}$ respectively. It showed positive growth regulation

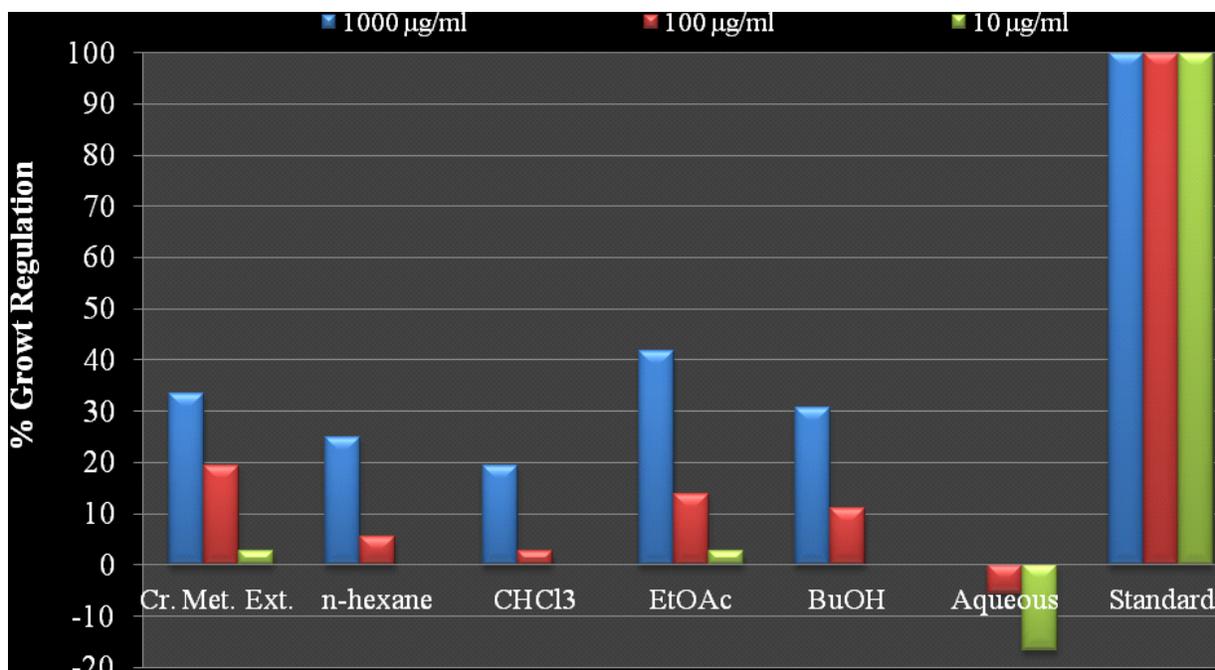


Figure 5. Phytotoxic activity of crude methanolic extract and various fractions of *L. cylindrica*.

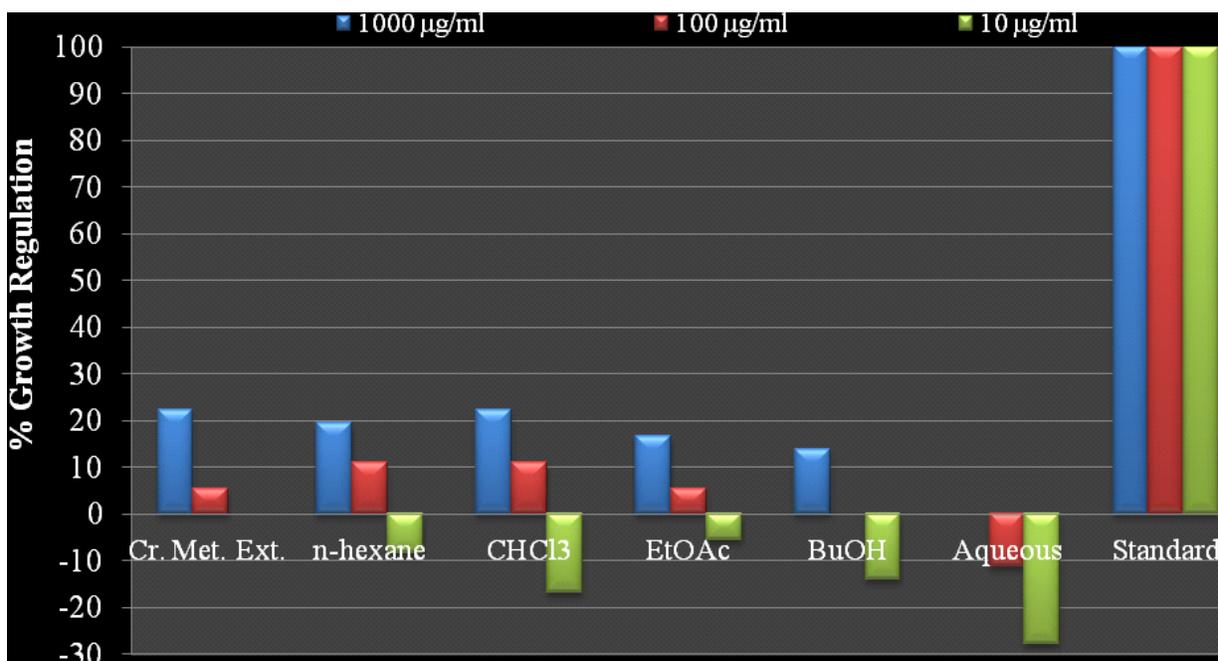


Figure 6. Phytotoxic activity of crude methanolic extract and various fractions of *M. charantia*.

of 8.33% at 10 µg/ml. The CHCl₃ fraction presented low growth regulation of 22.22 and 11.11% at 1000 and 100 µg/ml respectively. It showed positive growth regulation of 16.66% at 10 µg/ml. The EtOAc fraction displayed low growth regulation of 16.66 and 5.5% at 1000 and 100 µg/ml, respectively. It showed positive growth regulation

of 5.5% at 10 µg/ml. The butanol fraction presented low activity 13.88% at 1000 µg/ml. At 100 µg/ml, it was inactive and at 10 µg/ml presented positive growth regulation of 13.88%. The aqueous fraction presented no growth inhibition at 1000 µg/ml, whereas at 100 and 10 µg/ml displayed growth promoting activity of 11.11 and

27.77%, respectively.

Conclusion

From the previous study it is concluded that both of the plants contains potent antifungal property as the BuOH fraction of *L. cylindrica* showed significant activity against *F. solani* (85%) and *T. longifusus* (80%) while the crude methanolic extract and EtOAc fraction of the plant showed good linear growth inhibition against *M. canis* (70%). The *n*-hexane fraction of *M. charantia* showed significant inhibition against *T. longifusus* (85%), EtOAc fraction against *A. flavus* (80%) and BuOH fraction against *C. glaberata* (80%).

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