Activity of Some Medicinal Plants Against Phytopathogenic Fungi

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Abstract- Crude extracts of 17 medicinal plants were examined for their antifungal properties against phytopathogenic fungi. Hexane, chloroform and methanol extracts were tested using disc diffusion method at concentrations varying from 1.25 to 5 mg/disc. Among these, hexane extract of Blumea mollis was the most effective against Aspergillus flavus with zone of inhibition of 23mm at 5mg/disc concentration. The Minimal inhibitory concentration (MIC) of B. mollis hexane extract against A. flavus, A. niger and Botrytis cinerea were 0.623, 0.825 and 0.622 mg/ml respectively. Terpenoids, saponins, anthraquinones and anthocyanins were detected in the hexane extract of B. mollis. Subsequently, GC-MS analysis of the extract revealed the main constituent to be cycloheptane (34.21%). This could have potential use as a fungicide against phytopathogens.

Keywords: Phytopathogenic fungi, plant extract, disc diffusion, MIC, GCMS

I. INTRODUCTION

Crop production faces many problems among which fungal diseases are major cause of yield loss throughout the world resulting in excessive economic burden [1]. Yield losses caused by pathogens, animals and weeds altogether range between 20 and 40% in global agricultural productivity [2]. Though majority of the plant diseases are caused by phytopathogenic fungi, some diseases are also caused by bacteria, nematodes and viruses [3]. Fungi are known to infect the plants during the developmental stages and also at post-harvest period [4]. Degradation of grains, vegetables and fruits caused by pathogenic fungi may lead to loss of entire product. Involvement of Aspergillus sp. along with variety of other fungi like Botrytis cinerea and Fusarium sp.) in the accumulation of fungal toxins in seed and loss of nutritional quality has been reported [5]. Synthetic chemical agents are heavily used to control the phytopathogenic fungi, which results in resistance development in the pathogens and accumulation of chemicals in the environment [6,7]. Synthetic chemicals are also toxic to human beings and cause carcinogenicity and teratogenicity [8]. Numerous countries in the yester years have proscribed the agricultural pesticides due to their toxicity to non-targets such as humans [9]. So there is a need for plant-based compounds for ecofriendly applications to control the crop damage caused by fungi, bacteria, nematodes and other organisms. Plants produce secondary metabolites such as flavonoids, alkaloids, terpenoids etc., for aforementioned reason as well as other uses as antimicrobials, insecticides, nematicides along with other activities [10, 11, 12, 13, 14, 15, 16]. Many medicinal plants have fungicidal properties [17] against different fungal species including phytopathogenic fungi. Blumea mollis is an aromatic annual herb. It is a weed commonly found in India, Sri Lanka and Myanmar [18]. The leaf has been traditionally used for skin diseases and the boiled herb has been used to treat diarrhea [19]. The whole plant mixed with garlic is used to treat leucorrhoea [20]. The present study was carried out to investigate the inhibitory effects of different organic solvent extracts of 17 medicinal plants against 11 phytopathogenic fungi and minimal inhibitory concentration was found out for the most effective extract.
II. MATERIALS AND METHODS

Plant collection and identification
Seventeen medicinal plants were collected from different places in the state of Tamil Nadu, India (Table 1). All the plant species were collected during flowering stage in the month of March to May 2016. The taxonomical identification of the plant species was performed by taxonomist Dr. Pandikumar, Entomology Research Institute (ERI), Loyola College, Chennai. Voucher specimens were deposited in the herbarium at ERI.

Preparation of crude extract
The plant materials were shade-dried at room temperature and powdered using an electric blender. The powdered plant materials (100 g) were sequentially extracted with hexane, chloroform, and methanol. After 72 h of soaking of the plant material in each solvent, the extract was filtered through Whatman filter paper using a vacuum. Solvent in the extract was removed using a rotary vacuum evaporator at 45-55°C and the crude extracts were stored at 4°C in the refrigerator for further bioassay.

Fungal culture and spore collection.
Eleven phytopathogens namely Alternaria alternata, Aspergillus flavus, Aspergillus niger, B. cinerea, Bipolaris oryzae, Curvularia lunata, Curvularia oryzae, Fusarium solani, Fusarium oxysporum, Macrophomina sp and Trichoderma sp were collected from the Department of Plant Pathology, Annamalai University, Tamil Nadu, India. All the fungal cultures were grown in Potato dextrose agar medium for 7-10 days and spore suspension was filtered with sterile muslin cloths; conidia spores were collected and spore suspension was adjusted to 2-10^5 spores/ml using haemocytometer [21].

Antifungal susceptibility test
Antifungal activity of 17 plants was screened by following the kirby bauer disk diffusion method [22]. Sterile Potato Dextrose Agar (20 ml) (PDA, HiMedia) was taken in petri plates and the agar was allowed to solidify for 5 minutes; the fungal spore suspension was swabbed uniformly on the agar. Different concentrations of plant extracts viz., 1.25, 2.5 and 5 mg/disc were used [23] for diffusion method and the reference control, Hexaconazole (5 mg/ml) was used as positive control for comparison. The extract was dissolved in water + 2% Dimethyl sulfoxide (DMSO) and was loaded on separate sterile discs (6mm diameter). The extract-loaded discs were placed on the surface of agar medium and the extract was allowed to diffuse for 5 minutes. The plates were kept in incubator at 27°C for 48 to 96 h. At the end of incubation, inhibition zones formed around the discs were measured with a Vernier caliper in millimeter (Antibiotic zone scale). This study was performed in triplicate.

Minimal inhibitory concentration
Minimum Inhibitory Concentration (MIC) was found out using microdilution technique using standard method (CLSI, 1998) [23]. The extracts were dissolved in water + 2% Dimethyl Sulfoxide (DMSO). The initial test concentration of the extract was 1 mg/ml. Hexaconazole and carbendazim were used as standard drugs. Test concentration was serially diluted (5 mg to 0.1) in a 96 well plate. Each well was inoculated with 10 μl of spore suspension containing 10^4 spores/ml of fungi. The plates were incubated at 27°C for 3 to 5 days. MIC was performed as the lowest extract concentration, showing no visible fungal growth after incubation time. 10 μl of tested broth was placed on the sterile PDB medium for fungi and incubated at respective temperature. The MIC for fungi was determined as the lowest concentration of the extract inhibiting the visual growth of the test fungal cultures on the 96 well plate.

Preliminary phytochemical analysis of B. mollis extract
Qualitative phytochemical analysis of the active plant extract was done following the method of Yadav et al. (2014) [24].

The GCMS analysis
The GCMS analysis of hexane extract of B. mollis leaves was done using Agilent Technologies GC systems with model name GC-7890A/MS-5975C (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column 30 m in length × 0.25mm in diameter × 0.25 μm in thickness film. Spectroscopic finding by GCMS involved an electron ionization system which consumed high energy electrons (-70eV). The carrier gas of pure helium gas (99.995%) was used with flow rate of 1.0 ml per min. The oven program as follows initial temperature was set at 50°C, held at 1 min; the second step was at 170 °C for 0 min and the final step was at 300 °C for 10 min. 2 μl of the prepared extract 1% diluted with respective solvent was injected in a splitless mode with average velocity of 36.445 cm/sec. Comparative quantity of the chemical compounds present in hexane extract of B. mollis was expressed as percentage based on peak area formed in the chromatogram.
III. RESULTS AND DISCUSSION

Table 1 shows the details of botanical name, family, local name, collection time and yield of crude extracts. The result of the antifungal screening of the crude extracts of all plant species is shown in table 2. Among the 17 plants screened, the B. mollis hexane extract showed promising activity against tested fungi. The hexane extract of B. mollis showed (Figure 1) maximum zones of inhibition against A. flavus (23 mm), A. niger (18.33 mm), B. cinerea (18.33 mm), C. oryzae (16.33 mm), C. lunata (20.66 mm), F. solani (17.33 mm) and F. oxysporum (14.33 mm). The chloroform extract showed moderate activity against F. solani, B. cinerea and C. oryzae (Table 3). The MIC of B. mollis hexane extract was low against A. flavus (0.623 mg/ml), A. niger (0.825 mg/ml), B. cinerea (0.622 mg/ml), C. oryzae (1.25 mg/ml), C. lunata (0.821 mg/ml) and F. solani (1.15 mg/ml) (Table 4).

The commercial drug Hexaconazole showed MIC values of against A. flavus, B. cinerea 1.25 ± 1 mg/ml, A. niger (0.622 mg/ml) and C. oryzae, C. lunata, F. solani F. oxysporum did not show any activity, even at 5mg/ml concentration. Fungicide Bevistine showed MIC value of (1.25 ± 0 mg/ml) against A. flavus, B. cinerea, C. oryzae followed by A. niger, C. lunata, F. oxysporum (0.622 mg/ml) and F. solani (0.312 ± 0 mg/ml). The chemical constituents were identified using GCMS (Figure 2). The results showed that 27 compounds were detected (Table 6) in the hexane extract of B. mollis among which three compounds namely Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- (18.24%), Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl(34.21%) and Caryophyllene oxide (26.73%) were found as major components.

Fungal diseases cause severe growth reduction and yield loss in many agricultural, ornamental and horticultural crops. Fungal spores easily spread through air, water and soil and infect other plants. At present, ecofriendly biochemicals are more preferred to control plant pathogens to avoid environmental contamination, non-target effects and side effects in humans. Medicinal plant extracts are promising sources of biologically active chemicals that may be used to control phytopathogenic fungi [25, 26]. In the present study, 51 crude extracts from 17 medicinal plants were screened against important phytopathogenic fungi. B. mollis hexane extract showed the most potential activity against many pathogenic fungi. B. mollis is a weed; but has many medicinal properties. Native people of in Andhra Pradesh in India are using B. mollis to treat hepatotoxicity, asthma and dropsy [18]. The methanol extract of B. mollis had hepatoprotective activity in wistar rats [27]. The water extract B. mollis showed anti-inflammatory, antioxidant activity and anticancer activities [28].

B. mollis possessed antifungal activity against 11 phytopathogens. All other plants, tested in this study did not show antifungal activity against the tested pathogenic fungi. Hexane extract presented maximum zone of inhibition of 23mm against A. flavus. Hexane extract of B. mollis used in the present study was found to be the better antifungal agent than many previously reported plant extracts. For example Chandrasekarar and Venkatesalu [29] tested the methanol and aqueous extracts of Syzygium jambolanum seed against 9 fungal pathogens at 1mg/ml concentration. The extract presented the highest zone of inhibition of 15 mm against A. flavus and A. fumigatus. Ethanol and aqueous extracts of Acalypha wilkesiana showed maximum zone of inhibition of 10 and 15 mm respectively at 30mg/ml dose against A. flavus [30]. Ethanol extract of Nymphaea nouchali seeds presented 10mm, 10mm and 11mm zones of inhibitions at 1mg/ml against A. niger, Penicillium sp. and Curvularia sp. respectively [31]. Similarly, the methanol extract of the bark and leaves of Acacia nilotica showed 12 mm zone of inhibition against A. flavus and leaf extract of Ziziphus mauritian recorded 11 mm zone of inhibition at 10mg/ml [32]. In our study, the MIC value of hexane extract of B. mollis was recorded as 0.625 mg/ml, which clearly showed its potential antifungal activity against A. flavus, A. niger and B. cinerea. Similar study by Breda et al. (2016) [4] reported that the ethanol extracts of Correio brasiiliense (pequi) leaves and fruit peel were active against Alternaria alternata, Alternaria solani and Venturia pirina with MIC ranging between 350 and 1000 μg/mL. In another study, the root part of Hypochaeris radicata presented MIC values of 200 and 600μg/mL against A. niger and Mucor sp. respectively [33]. The ethanol extract of Rhus muelleri showed growth inhibition against F. oxysporum at MIC value of 11.793 ppm [34], which was very high compared to the MIC value of B. mollis hexane extract in the present study.

The GCMS study showed that hexane extract of B. mollis had three major compounds namely Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- (18.24%), Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl(34.21%) and Caryophyllene oxide (26.73%). Previously Senthilkumar et al. (2008) [35] reported that essential oil of B. mollis contained linalool (19.43%) and γ-elemene (12.19%) as major ingredients. In a previous study caryophyllene oxide has been reported as an antifungal agent [36].

IV. CONCLUSION AND FUTURE SCOPE

In the present B. mollis hexane extract showed promising activity against major phytopathogenic fungi at very low concentration. Since B. mollis is already used as medicinal plant, it can be included in the control of plant pathogenic fungi. B. mollis is a weed and easily available one. Secondary metabolites of plant origins have noticeable impressions on many research
fields and added benefit of producing inexpensively. Finding of more such products is a boon for eco-friendly crop management, in turn they ameliorate the redundancy of synthetic chemicals. Furthermore extensive research is required to identify much more bioagents antagonistic to phytopathogens.

Figure 1. Antifungal activity of hexane extract of *B. mollis* against phytopathogenic fungi

Hexane extract: H1- 1.25 mg, H2- 2.50 mg, H3- 5 mg. Chloroform: Ch1- 1.25 mg, Ch2- 2.50 mg, Ch2- 5mg. C - control (hexaconazol) 5mg

Figure 2) GCMS chromatogram of hexane extract of *B. mollis* showing the major compound
Table 1: List showing the names, parts used, place and month of collection and voucher specimen number of different medicinal plants used in the antifungal screening experiment

| Botanical name and family | Local name | Collection place | Collection stage of plant and month | Plant part used | Voucher specimen number |
|---------------------------|------------|------------------|------------------------------------|-----------------|------------------------|
| *Anisomeles malabarica* (Linn.) Lamiaceae | Pei miratti | Pitchavaram mangrove forest | Flowering stage; May | Whole plant | ERISS-01 |
| *Atlantia monophylla* (Linn.) Rutaceae | Kattu elumichai | Pitchavaram mangrove forest | Fruiting stage; July | Leaf | ERISS-02 |
| *Blumea lacera* (Linn.) Asteraceae | Kaatsumullangi | Pitchavaram mangrove forest | Flowering stage; June | Leaf | ERISS-03 |
| *Blumea mollis* (D.Don) Merr. Asteraceae | Soft blumea | Cuddaloredistrict; (Akkravaram lake forest) | Flowering stage; May | leaf | ERISS-04 |
| *Clerodendrum inerme* (Linn). Lamiaceae | Sangamkuppi | Loyola college campus | Flowering stage; June | Leaf | ERISS-05 |
| *Croton bonplandianum* (Linn). Euphorbiaceae | Rail poondu | Cuddalore district; (Akkravaram lake forest) | Fruiting stage; May | Arial part | ERISS-06 |
| *Cordia dichotoma* (Linn). Boraginaceae | Kalvirusu | Avadi | Fruiting stage; June | Leaf | ERISS-07 |
| *Murraya paniculata* (Linn). Rutaceae | Konjipazham | Cuddalore district; (Akkravaram lake forest) | Fruiting stage; June | Leaf | ERISS-08 |
| *Mollugo cerviana* (Linn). Molluginaceae | Parpadagum | Cuddalore district; (Akkravaram lake forest) | Flowering; June | whole plant | ERISS-09 |
| *Pedilanthus tithymaloides* (Linn). Euphorbiaceae | Kannaadikkalli | Avadi | Dormant stage; June | Leaf | ERISS-10 |
| *Quisqualis indica* (Linn) Combretaceae | Iranganmalli | Avadi | Flowering | Leaf | ERISS-11 |
| *Streblus asper* (Lour) Moraceae | Paraimaram | Loyola college campus | Flowering stage; June | Leaf | ERISS-12 |
| *Sphaeranthus indicus* (Linn) Asteraceae | Karanthai/kotta karanthai | Cuddalore district; (Akkravaram lake forest) | Flowering stage; May | Aerial part | ERISS-13 |
| *Secamo neemetica* (Retz) | Siruathankodi | Cuddalore | Dormant stage; | Aerial | |

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### Table 2: Antifungal activity of hexane, chloroform and methanol extracts of 17 medicinal plant extracts against phytopathogenic fungi by disc diffusion method.

| Plant name               | solvent   | Concentration (mg/disc) | Zone of inhibition (mm) |
|--------------------------|-----------|-------------------------|-------------------------|
|                          |           | A.a  | A.f | A.n | B.c | B.o | C.o | C.l | F.s | F.o | M sp | T sp |
| *A. malabarica*          | Hexane    | 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | 9   | 8   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          | Chloroform| 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | 8   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | -   | 10  | -   | -   | -   | 8   | -   | -   | -    | -    |
|                          | Methanol  | 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
| *A. monophylla*          | Hexane    | 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | -   | 9   | 8   | -   | -   | -   | -   | -   | -    | -    |
|                          | Chloroform| 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          | Methanol  | 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 2.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                          |           | 5     | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
| *B. lacera*              | Hexane    | 1.5   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -    |
|                | Chloroform | Methanol |        |       |        |       |        |       |       |       |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
|----------------|------------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **B. mollis**  | Chloroform | Methanol | B. mollis Hexane | 1.5 | -     | 15    | 15    | 15    | -     | 12    | 16    | 10    | 11    | -     | -     | 16    | 10    | 11    | -     | -     | 16    | 10    | 11    | -     |
|                | 2.5        | -       | 18    | 17    | 17    | -     | 14    | 18    | 13    | 13    | -     | -     | 2.5   | -     | 12    | 12    | 11    | -     | 13    | 13    | 13    | -     |
|                | 5          | -       | 23    | 20    | 18    | -     | 16    | 20    | 16    | 16    | -     | -     | 5     | -     | 14    | 15    | 13    | -     | 12    | 14    | 15    | 15    | -     |
| **C. inerme**  | Chloroform | Methanol | C. inerme Hexane | 1.5 | -     | -     | -     | -     | -     | -     | -     | -     | -     | 1.5   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
|                | 2.5        | -       | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | 2.5   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
|                | 5          | -       | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | 5     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| **C. bonplandianum** | Chloroform | Methanol | C. bonplandianum Hexane | 1.5 | -     | -     | -     | -     | -     | -     | -     | -     | -     | 1.5   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
|                | 2.5        | -       | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | 2.5   | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
|                | 5          | -       | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | 5     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
|                |          | 1.5 | 2.5 | 5   | 10 | 20 | 50 | 100 |
|----------------|----------|-----|-----|-----|----|----|----|-----|
| Methanol       | C. dichotoma | Hexane | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Chloroform     |           |      | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Methanol       | M. paniculata | Hexane | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | 8   | -  | -  | 9  | 9   |
| Chloroform     |           |      | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | 9  | 9   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Methanol       | M. cerviana | Hexane | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Chloroform     |           |      | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Methanol       |           |      | 1.5 | -   | -  | -  | -  | -   |
|                |           |      | 2.5 | -   | -  | -  | -  | -   |
|                |           |      | 5   | -   | -  | -  | -  | -   |
| Species      | Extraction Solvent | 1.5 | 2.5 | 5   | Chloroform | 1.5 | 2.5 | 5   | Methanol | 1.25 | 2.5 | 5   | 2.5 | 5   | 5   | 2.5 | 5   | 5   | 1.5 | 2.5 | 5   | 8   | 9   | Chloroform | 1.5 | 2.5 | 5   |
|--------------|--------------------|-----|-----|-----|------------|-----|-----|-----|---------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|-----|
| *P. tithymaloides* | Hexane            | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Chloroform         | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Methanol           | 1.25| -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| *Q. indica*   | Hexane            | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Chloroform         | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Methanol           | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| *S. asper*    | Hexane            | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Chloroform         | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Methanol           | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| *S. indicus*  | Hexane            | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 2.5 | 8   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               |                    | 5   | 9   | -   | -   | 8   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Chloroform         | 1.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |

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|       | S. emetica | T. purpurea | T. asiatica |
|-------|-----------|-------------|-------------|
|       | Hexane    |             |             |
|       | 1.5       | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
| Methanol | 1.5     | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
| Chloroform | 1.5  | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
| Methanol | 1.5     | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
| Chloroform | 1.5  | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
| Methanol | 1.5     | 1.5         | 1.5         |
|       | 2.5       | 2.5         | 2.5         |
|       | 5         | 5           | 5           |
|       | 5         | 9           | 9           |
|       | 8         | 8           | 8           |
|       | 5         | 5           | 5           |

There are values representing concentrations or other measurements for each of the plant species tested with different solvents.
Table 3: Antifungal activity of hexane and chloroform extracts of *B. mollis* by disc diffusion method

| Extract  | Conc.(mg/disc) | Zone of inhibition (mm) |
|----------|----------------|-------------------------|
|          | Af             | An                      | Bc              | Co              | Cl              | Fs              | Fo              |
| Hexane   | 1.5            | 15.33 ± 0.77            | 15 ± 0          | 15.33 ± 0.57    | 12 ± 1          | 16.33 ± 0.57    | 14 ± 0          | 11.33 ± 0.57    |
|          | 2.5            | 18.33 ± 0.57            | 16.66 ± 0.57    | 17 ± 0          | 13.66 ± 0.57    | 17.66 ± 0.57    | 15.33 ± 0.57    | 12.66 ± 0.57    |
|          | 5              | 23 ± 1                  | 18.33 ± 0.57    | 18.33 ± 0.57    | 16.33 ± 0.57    | 20.66 ± 0.57    | 17.33 ± 0.57    | 14.33 ± 0.57    |
| Chloroform | 1.5        | 11.66 ± 1.15            | 10.33 ± 0.57    | 10.66 ± 0.57    | -               | 12.33 ± 0.57    | 11.33 ± 0.57    | 11.66 ± 0.57    |
|          | 2.5            | 12 ± 0                  | 12.33 ± 0.57    | 11.66 ± 0.57    | -               | 14.33 ± 0.57    | 13.33 ± 0.57    | 12.33 ± 0.57    |
|          | 5              | 13.66 ± 0.57            | 14.66 ± 0.57    | 13 ± 0          | 11.66 ± 0.7     | 15 ± 1          | 14.66 ± 0.57    | 14 ± 0          |
| Reference Control (Hexaconazole) | 5 | 11 ± 1 | 12 ± 0 | 10 ± 1 | 11 ± 0 | - | - | - |

* A.a – A. alternata, Af – A. flavus, An – A. niger, B.c-B. cinerea, Bo-B. oryzea, Co- C. oryzea, Cl- C. lunata, Fs – F. solani, Fo- F. oxysporum, - no activity.

A.f-A. flavus, A.n- A. niger, B.c-B. cinerea, C.o- C. oryzea, C.l- C. lunata, F.s – F. solani, F.o – F. oxysporum, *Macrophomiana* sp, *Trichoderma* sp, - no activity. Values are mean ± standard error.

Table 4: MIC values (Mean± SE) of hexane and chloroform extracts of *B. mollis* against phytopathogenic fungi.

| Microorganism   | Minimal inhibitory concentration |
|-----------------|---------------------------------|
|                 | Hex extract mg/disc | Hexaconazole* (mg/ml) | Carbendazim * (mg/ml) |
| *A. flavus*     | 0.625 ± 1 | 1.25 ± 0 | 1.25 ± 0 |
| *A. niger*      | 0.625 ± 0 | 0.625 ± 0 | 0.625 ± 0 |
| *B. cinerea*    | 0.625 ± 2 | 1.25 ± 0 | 1.25 ± 0 |
| *C. oryzea*     | 1.25 ± 1 | - | 1.25 ± 0 |
| *C. lunata*     | 0.3125 ± 0 | 5 ± 0 | 0.625 ± 0 |
| *F. solani*     | 1.25 ± 2 | - | 0.312 ± 0 |
| *F. oxysporum*  | >5 ± 1 | - | 0.625 ± 0 |

- No activity; *commercial fungicidal compounds,
Table 5: Preliminary phytochemical analysis for \textit{B. mollis} extracts

| Test            | Hexane | Chloroform | Methanol |
|-----------------|--------|------------|----------|
| Tannins         | -      | -          | +        |
| Flavonoids      | -      | +          | -        |
| Terpenoids      | +      | +          | +        |
| Saponins        | +      | +          | +        |
| Steroids        | -      | -          | +        |
| Carbohydrates   | -      | +          | +        |
| Glycosides      | +      | -          | -        |
| Coumarins       | -      | -          | -        |
| Alkaloids       | -      | +          | +        |
| Proteins        | -      | +          | -        |
| Anthraquinones  | +      | +          | +        |
| Anthocyanins    | +      | -          | +        |

+ present; - absent

Table 6: GCMS analysis of hexane extract of \textit{B. mollis}

| Peak no | compound name                                                                 | Retention time | Area of percentage |
|---------|-------------------------------------------------------------------------------|----------------|--------------------|
| 1       | 6-Tridecene, (Z)-                                                             | 4.332          | 2.29               |
| 2       | 6-Tridecene, (Z)-                                                             | 5.228          | 3.61               |
| 3       | beta.-curcumene                                                              | 5.265          | 2.85               |
| 4       | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- | 5.326          | 4.19               |
| 5       | 4-tert-Butylcatehol, dimethyl ether                                           | 5.409          | 1.15               |
| 6       | Isocaryophyllene                                                             | 5.529          | 18.24*             |
| 7       | 7-Pentadec-5-yne, (Z)-                                                        | 5.725          | 1.82               |
| 8       | 8,11-Octadecadiynoic acid, methyl ester                                       | 5.824          | 1.92               |
| 9       | Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl          | 5.928          | 34.21*             |
| 10      | Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]- | 5.976          | 4.24               |
| 11      | 4-epi-cubedol                                                                | 6.088          | 5.44               |
| 12      | 6-Tridecene, (Z)-                                                             | 6.47           | 4.04               |
| 13      | Caryophyllene oxide                                                          | 6.626          | 26.73*             |
| 14      | Caryophyllene oxide                                                          | 6.827          | 2.61               |
| 15      | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-                       | 7.028          | 5.15               |
| 16      | cis-5,8,11,14,17-Eicosapentaenoic acid                                        | 7.294          | 2.36               |
| 17      | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-                       | 7.391          | 1.49               |
| 18      | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-                       | 7.456          | 1.06               |
| 19      | 8,11-Octadecadiynoic acid, methyl ester                                        | 7.675          | 1.56               |
| 20      | cis-Z.-alpha.-Bisabolene epoxide                                              | 7.802          | 3.32               |
| 21      | 1-Eicosano                                                                   | 8.336          | 2.88               |
| 22      | Butyrylthioacetic acid, ethyl ester                                           | 11.393         | 9.6                |
| 23      | Phytol                                                                       | 12.575         | 4.03               |
| 24      | Tetraacetyl-d-xylonic nitrile                                                 | 12.635         | 7.06               |
| 25      | Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, semicarbazone                   | 12.785         | 4.6                |
| 26      | 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl                            | 23.012         | 8.34               |
| 27      | Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl                          | 26.548         | 4.81               |

* - major compound
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Conflicts of Interest- The authors declare that there are no conflicts of interest

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