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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 burdens [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, teripinoids 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 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 vacuum. Solvent in the extract was removed using rotary vacuum evaporator at 45-55^{o}C and the crude extracts were stored at 4^{o}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^{o}C for 48 to 96 h. At the end of incubation, inhibition zones formed around the sterile disc 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 wereused as standard drugs. Test contration was serially diluted (5 mg to 0.1) in a 96 well plate. Each well was inoculated with 10 µl of spore suspension containing 104 spores/ml of fungi. The plates were incubated at 27° 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 Tec hnologies, 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* (23mm), *A.niger* (18.33mm), *B. cinerea* (18. 33mm), *C.oryzae* (16. 33mm), *C. lunata* (20.66mm), *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. oryzea*. (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. oryzea*, *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. oryzea* 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 Chandrasekaran 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*, *Penicllium* sp, and *Curvalaria* 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 *Zizphus 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. cinearae*. Similar study by Breda et al. (2016) [4] reported that the ethanol extracts of *Correio brasiliense* (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.

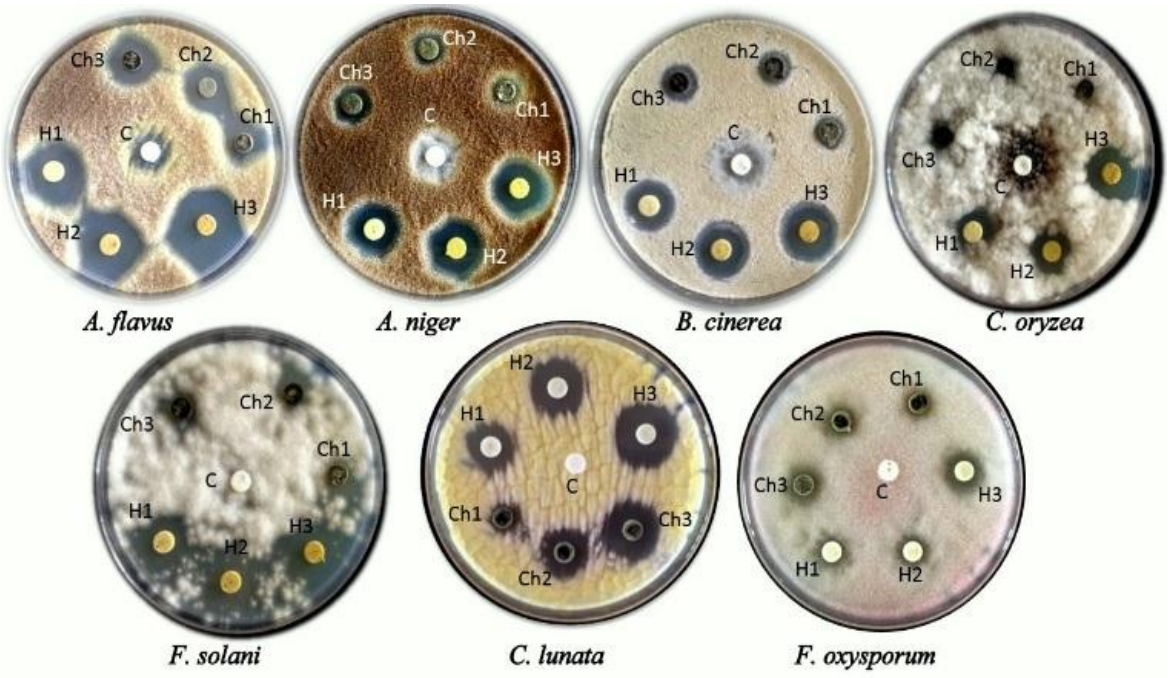

Figure 1. Antifungal activity of hexane extract of *B. mollis* against phytopathogenic fungi

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

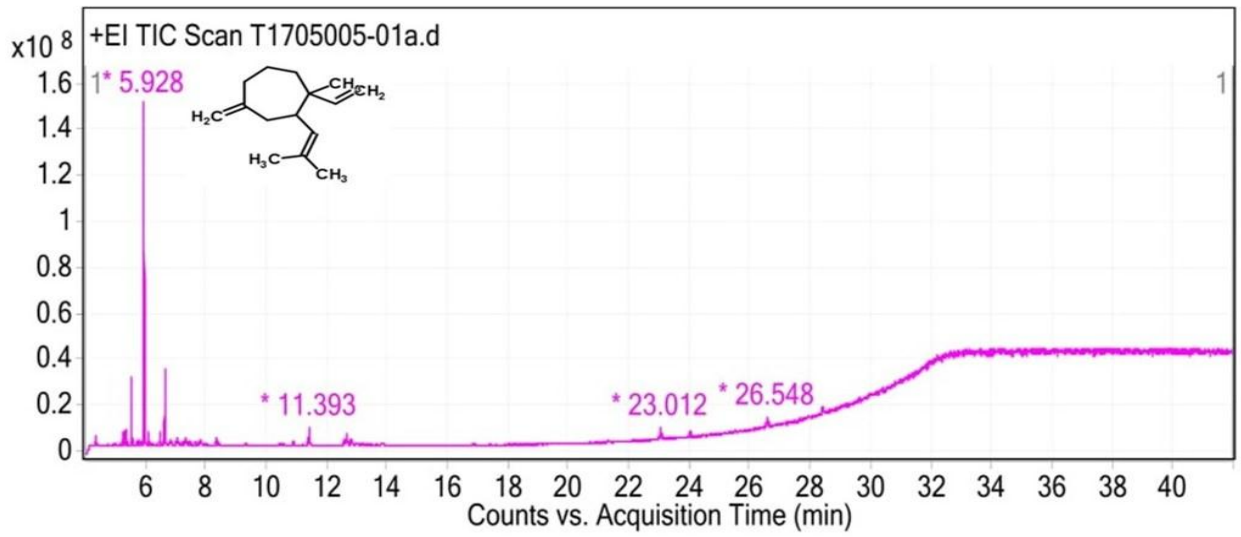

Figure 2) GCMS chromatogram of hexane extract of *B. mollis* showing the major compound

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	Kaatumullangi	Pitchavaram mangrove forest	Flowering stage; June	Leaf	ERISS-03
Blumea mollis (D.Don) Merr. Asteraceae	Soft blumea	Cuddaloredistric (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	Kannaadikkallli	Avadi	Dormant stage; June	Leaf	ERISS-10
Quisqualis indica (Linn) Combretacea	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	

Asclepiadaceae		district (Akkravaram lake forest)	June	part	ERISS-14
Tephrosia purpurea (Linn). Fabaceae	Kolinji	Pitchavaram mangrove forest	Dormant stage; July	Whole plant	ERISS-15
Toddalia asiatica (Linn). Rutaceae	Milakaranai	Cuddalore district (Akkravaram lake forest)	Flowering stage; June	Aerial part	ERISS-16
Wedelia calendulacea (Linn). Asteraceae	Ponnirachi	Cuddaloredistric (Akkravaram lake forest)	Flowering stage; July	Aerial part	ERISS-17

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	-	-	-	-	-	-	-	-	-	-	-

		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	8	-	-	9	10	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
B. mollis	Hexane	1.5	-	15	15	15	-	12	16	10	11	-	-
		2.5	-	18	17	17	-	14	18	13	13	-	-
		5	-	23	20	18	-	16	20	16	16	-	-
	Chloroform	1.5	-	11	10	10	-	–	11	11	12	-	-
		2.5	-	12	12	11	-	–	13	13	13	-	-
		5	-	14	15	13	-	12	14	15	15	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
C. inerme	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
C. bonplandianum	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-

		5	-	-	-	-	-	-	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
C. dichotoma	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
M. paniculata	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	8	-	-	-	-	-	-	-
		5	8	-	-	9	-	-	-	-	9	-	9
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	9	-	9	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
M. cerviana	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
	Methanol	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	-	-	-	-	8	
	Chloroform	1.5	-	-	-	-	-		-	-	-	-	_	

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

		5	-	-	-	9	8	-	-	-	-	-	-
W. calendulacea	Hexane	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5		-	-	-	-	-	-	-	-	-	-
	Chloroform	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5		-	-	-	-	-	-	-	-	-	-
	Methanol	1.5	-	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-
Standared control (Hexaconazole)		5	-	11	12	10	15	11	-	-	-	12	15

A.a. – A. alternata, *A.f-A. flavus*, *A.n- A. niger*, *B.c-B. cineriae*, *B.o-B. oryzea*, *C.o- C. oryzea*, *C.l- C. lunata*, *F.s –F. solani*, *F.o- F. oxysporum*, - no activity.

Table 3: Antifungal activity of hexane and chloroform extracts of *B. mollis* by disc diffusion method

Extract	Conc.(mg/ disc)	Zone of inhibition (mm)						
		A,f	A.n	B.c	C.o	C.l	F.s	F.o
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.f-*A. flavus*, A.n- *A. niger*, B.c-*B. cineriae*, 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 heaxane and chloroform extracts of *B. mollis* against phytopathogenic fungi.

Microorganism	Minimal inhibitory concentration		
	Hexane 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. cinearae	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 *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 *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-Butylcatechol, dimethyl ether	5.409	1.15
6.	Isocaryophyllene	5.529	18.24*
7.	7-Pentadecen-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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