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Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens

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Plants are reservoir of biological active compounds to combat various pathogens. Methanol extracts of five plants were tested for their antifungal activity against 10 phytopathogenic fungi and *Candida albicans* B017. Among all extracts, *Lawsonia inermis* showed greatest percent inhibition of mycelial growth of target fungi (76.47 - 87.77 %) followed by *Withania somnifera* (54.44 - 78.88 %). Other plant extracts also exhibited antifungal activity at varying percentage. Extract of *Lawsonia inermis* (1 %) increased the percent seed germination of chickpea (*Cicer aeritinum* L.) with 30 % and protected from *Fusarium oxysporum in vivo*. All extracts were analyzed for the detection of various secondary metabolites. Certain plant extracts contained alkaloids flavonoids, saponins, steroids and tannins. Saponin was found in all extracts. The mode of action of extracts was determined on cell wall and enzyme production of fungi. *Lawsonia inermis* inhibited the production of catalase in *Aspergillus niger* and *Fusarium oxysporum*. Thin layer chromatography was prepared in eight solvent systems. Out of which, toluene/ ethyl acetate (1:1) showed higher band separation in almost all extracts. Protein fractions of *Lawsonia inermis* and *Withania somnifera* exhibited four to five times more percent inhibition of mycelial growth of *Bipolaris oryzae* and *Colletotrichum lindemuthianum* than non-protein fractions. The active compounds were proteinaceous in nature or proteins and they are effective against plant pathogens.

Key words: antifungal activity, catalase, *Candida albicans* B017, *Lawsonia inermis*, protein fractions

Introduction

The fungi are major disease causing agents on plants and can lose up to 90% agricultural yield. Various systemic fungicides have been used to control the plant diseases. But due to indiscriminate use of synthetic fungicides, various important pathogens have been developed resistance to many of the currently

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available fungicides (Gangawane, 1990). Beside, this fungicide also polluting soil and water.

Sometimes the fungicide adversely affect on the non target organisms. Hence it is necessary to search new antifungal compounds as an alternatives, safe, eco-friendly, cheap to synthetic fungicides from plants, since they produce different secondary metabolites which perform defensive role in plants and protect the plants from their invaders. Plant extracts and essential oils has been investigated throughout the world for their antifungal activity against wide range of fungi (Ezzat, 2001; Abd-El-Khair and Hafez, 2006; Gupta *et al.* 2008). Therefore five methanol extracts were evaluated for antifungal activity against important pathogenic fungi.

Materials and methods

The healthy, infection free mature parts of plants were collected from Government Institute of Science campus, Aurangabad. Identities of plants species were authenticated by referring standard literature. Plant pathogens were isolated from various plants and soil. Whereas *Candida albicans* B017 was obtained from Department of Microbiology, Government Institute of Science, Aurangabad.

Preparation of crude extracts

Extracts were prepared in methanol at room temperature by simple extraction method (Deshpande *et al.,* 2004). Collected plant parts were shade dried and ground to a fine powder using grinder mixer. Dried powder of plant parts (10g) was mixed with 100 ml solvent in 250 ml conical flask. The flasks were plugged tightly with cotton and wrapped with papers. All conical flasks were kept on shaker for 24 h then it was allowed to stand for five hours to settle the plant materials. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 45 ^{o}C in vacuum evaporator to make the final volume 1/5th of the original volume. It was stored at 4 ^{o}C in airtight bottles for further studies.

Antifungal assay

Antifungal activity of plants was determined by food-poisoned technique (Schmitz, 1930). Standard extracts (5 ml) was mixed with 45 ml of sterilized PDA medium and transferred equally into two Petri plates. The media was allowed to solidify. The seven day old fungal culture disk of 6 mm diameter was taken and inoculated to the center of Petri plates containing plant

extracts in aseptic condition. Instead of PDA medium, without plant extracts served as control. The fungicide Dithane M-45 (0.2%) was used for comparison. All plats were incubated at 28 ± 2 ^{0}C and radial growth of colony was measured after seven day of incubation. Each test was performed in triplicate.

Effect of Withania somnifera leaves extracts on seed germination and seed born infection in vitro

The seeds of six commercial crop plants were obtained from local market of Aurangabad. All selected seeds were immersed in 10 % (v/v) extracts of *W. somnifera* for 20 min and equal number of seed was immersed in distilled water as a control. Both treated and untreated seeds were kept on moist blotter in 10 cm Petri plate. Number of seeds per plates varied with size of the seeds. In each case, 100 seeds were screened in duplicates. All the plates were incubated at 28± 2 ^{o}C. The percent of seed germination, seed borne infection of fungi, average length of radicle and plumule was recorded. Fungi which grown on the seeds were isolated, identified and cultures were maintained in PDA slants for further studies.

Effect of Lawsonia inermis extracts on seed germination and wilt of chickpea (Cicer aeritinum L.)

Gram seeds were immersed in 1 % extract for 2 h. Afterward, 200 ml spore suspension of *Fusarium oxysporum* which was isolated from gram seed mixed with soil in pots. For control, untreated seeds were sown. In each pot, 10 treated seeds were immersed in sterile distilled water and sown in pots containing *F. oxysporum* suspension. In each case 100 seeds were sown in 10 pots and kept under sunlight. The percent of seed germination was recorded at different intervals.

Minimum inhibitory concentration (MIC) of Lawsonia inermis extracts

MIC was determined by two fold dilution method against fungi (Murray *et al.,* 1995). The extract was serially diluted with sterile distilled water and was introduced in to potato dextrose broth (PDB) to final concentration as 12.5, 25, 50 and 100 µl/ml (v/v). Spore suspension (10 µl) of each target fungi was inoculated in the test tube containing PDB medium and incubated for 2-7 days at 28± 2 ^{o}C. The control tubes containing PDB medium were inoculated only with fungal suspension. Concentration at which no visible growth was observed considered as MIC.

Preliminary phytochemical analysis of crude extracts

Phytochemical analysis in crude plant extracts were detected by following procedure of Adu *et al.* (2007) and Egwaikhide and Gimba, (2007).

Mode of action

Effect of extracts on cell wall of fungi: For the action of extract on cell wall of fungi, modified sorbitol protection and morphology (SPAM) assay was followed the method of Dekshit and Desai, (2000). This assay consists of two bioassay plates of yeast malt agar (YMA) seeded with *Candida albicans* B017 one of which was supplemented with 0.8 M sorbitol as an osmo-protectant. The inoculums was prepared by growing *C. albicans* B017 in YM broth overnight and O.D. was adjusted to 1.0 at 600 nm. 50 µl of this inoculum was added. To 20 ml per plate of molten and cooled yeast malt agar and yeast malt agar containing 0.8 M sorbitol to prepare bioassay plate of 9 cm diameter. Three well of 8 mm diameter were punched using sterile cork borer in each plate. In each well, 50 µl of the test extract were introduced. The antibiotics griseofulvin 50 µg / ml was used for comparison. The plates were incubated at 37 ^{o}C for 24 h. The zone of inhibition was recorded after 24 h and expressed in mm. Each experiment was performed three times. Those extracts which showing reduced and hazy zone of inhibition in the sorbitol – supplemented plate, resulting from osmotic protection as against a clear zone of inhibition in the control plate due to lysis of *C. albicans* B017 cells was considered to have a cell wall active antifungal agent.

Effect of Lawsonia inermis on enzyme secretion in *Aspergillus niger* and *Fusarium oxysporum*: Qualitative tests of enzymes were performed. In sterilized conical flasks of 250 ml capacity, 100 ml glucose nitrate media was poured. Then 7 day old cultures of fungi were inoculated and kept for 7-14 days at 28 ^{o}C for incubation. After incubation the culture filtrate was obtained using Whatman no. 1 filer paper.Test for amylase:- In each test tube, 2 ml of 0.1% starch solution was taken and it was kept for 24 h at 25 ^{o}C. The presence or absence of starch was treated by adding a drop of I_2KI solution to each test tube.Test for protease (Birette test):- In test tube, 2 ml of culture filtrate was taken. In it 2 ml of 1% gelatin solution was added. The tubes were incubated at 25 ^{o}C for 24 h. After 24 h in each test tube 2 ml of 6 N NaOH and 0.5 ml of $CuSO_4$ solution was added. Deep violet to blue colouration was strong positive test for proteins while light blue colour indicates that enzyme protease has hydrolyzed protein partially. Test for catalase:- In test tube, 4 ml culture filtrate of fungi was taken and 1 ml of dilute H_2O_2 (30:1) with water was added to this

tubes. The evolution of oxygen bubbles indicates the presence of enzyme.Test for dehydrogenase:- Culture filtrate (4 ml) of fungi was taken in sterile test tube to each of this test tube 1 ml of (0.5 %) 2, 3, 5 – triphenyl tetrazolium chloride was added. The formation of red colour usually within 15-20 min indicates the presence of dehydrogenase.

Separation of crude plant extracts into protein and non-protein fractions and their antifungal activity

The two most effective plant extracts were selected and separated into protein and non-protein fractions by ammonium sulphate precipitation. These extracts were centrifuged at 3000 rpm for 15 min. The supernatant solution was treated with ammonium sulphate (767 g/l) to precipitate the protein. The precipitated proteins were centrifuged at 10,000 rpm for 10 min. The supernatant contained the non-protein fractions. The pellet containing the protein fraction was suspended in 0.1 M phosphate buffer (pH 7.0) and dialyzed against phosphate buffer to remove the salts. Both protein and non-protein fractions were assayed for their antifungal activity against two fungi using food poisoned technique.

Thin layer chromatography (TLC)

TLC was carried out for these extracts on 10 X 20 cm aluminum sheets coated with 0.2 mm thickness Silica gel 60 (Merck). Each plant extracts (10 µl) was applied on TLC plate at equal distance with the help of micropipette. The extracts loaded plates were kept in chromatography chamber. The following solvent systems were tested to obtain the best separation of crude plant extracts. Chloroform/methanol (1:1), toluene/ethyl acetate (1:1), ethyl acetate/acetic acid/formic acid/water (50:5.5:5.5:13), ethyl acetate/methanol/water (40:5.4:5), Butanol/ethanol/water (5:1:2), chloroform/mathanol (95:5), n-butanol/acetic acid/water (4:1:5) and chloroform/ methanol/water (64:50:10). The developed chromatogram was observed under visible, UV light and after being exposed to different reagent such as Dregendroff's reagent, Mayer's reagent, Wagner's reagent, iodine vapours, ammonium vapours, etc. The R_f values of the separated spots were calculated.

Results

All extracts showed antifungal activity at varying percentage (Table 1). Percent inhibition of mycelial growth was ranging from 37.25- 87.77%. The highest percent inhibition of mycelial growth was recorded in *L. inermis* against

F. moniliforme and *B. oryzae* whereas the lowest one was observed in *Datura metel* against *M. phaseolina*. Almost all extracts exhibited greater antifungal activity against target fungi as compared to that of synthetic fungicide Dithane M-45. Overall *L. inermis* and *W. somnifera* were significantly inhibited mycelial growth of fungi. Extract of *W. somnifera* (10 %) inhibited seed germination of some tested crops seeds. While the incidence of microbial infection was reduced or controlled after the treatment of extracts (Table 2). Redicle and plumule length of seeds was also affected by extract (Table 3). After treatment of seeds with 1 % extract of *L. inermis*, 80 % seeds of chickpea were germinated in pots while in control pots only 50 % seeds germination was observed. The percent of seed germination in treated seeds was 30 % more than untreated seeds by reducing the infection of *F. oxysporum.* Among all tested plant extracts *L. inermis* was found to be most potent one. Therefore only *L. inermis* extract was selected for the determination of MIC. It showed variation in MIC against target fungi (Table 4). Fifty percent fungi were totally inhibited at 100 µl/ml (v/v) concentration of extract. The fungal genera *Colletotrichum capsici* was found to be more resistant to extract. Some plant extract showed positive test for alkaloids, flavonoids, saponinns, steroids and tannins (Table 5). Among all secondary metabolites, saponin was present in all extracts and most abundant one. Extract of *D. metel* and *W. somnifera* contained highest number of secondary metabolites. In modified sorbitol protection and morphology (SPAM) assay, none of the extract showed hazy and reduced zone of inhibition in treated plate containing plant extract and sorbitol (Table 6), though the extracts were not acting on cell wall of fungi, but effective against *C. albicans* B017 which are pathogenic to men. While antibiotics greseofulvin did not revealed zone of inhibition against *C. albicans* B017. Extract of *L. inermis* altered the enzyme secretion of both the fungi (Table 7). Production of catalase in both the fungi was inhibited by tested extract. It suggests that, extract interfere with metabolic activity of fungi and inhibited catalase production. With a view to identify the nature of active antifungal compound, crude extract of most effective plant *L. inermis* and *W. somnifera* were separated into protein and non-protein fractions. All the fractions were separately tested against *C. lindemuthianum* and *B. oryzae*. Protein fractions of both the plants revealed four to five time greater inhibition of mycelial growth of *C. lindemuthianum* and *B. oryzae* than non-protein fractions (Table 8). It indicates that the active compound might be proteinaceous in nature or protein.

Among eight solvent system tested, toluene/ethyl acetate (1:1) exhibited high band separation in TLC with almost all extracts (Table 9). In all plants, *L. inermis* revealed highest number of bands while *B. racemosa* was lowest one which represent particular compound. There were some similarities in the

chemical composition in different plant extracts because they showed same R_f values and colour.

Table 1. Antifungal activity of methanol extracts (10% v/v) of plants on various fungi.

Fungi	% inhibition of mycelial growth over control in extracts					
	LI	**WSL**	**DML**	**DSL**	**BRSB**	**D.M-45**
Colletotrichum capsici	83.58	74.62	68.65	76.11	53.73	17.91
Colletotrichum lindemuthianum	81.81	69.69	69.69	42.42	50.00	39.39
Fusarium moniliforme	87.77	54.44	50.00	53.33	52.22	37.77
Alternaria alternata	82.85	74.28	54.28	72.85	72.85	27.14
Bipolaris oryzae	87.77	55.55	63.33	52.22	71.11	78.88
Curvularia lunata	77.64	68.23	64.70	75.29	71.76	57.64
Rhizoctonia solani	81.11	78.88	75.55	62.22	60.00	43.33
Macrophomina phaseolina	76.47	72.54	37.25	64.70	43.13	41.17
Pyricularia oryzae	85.44	65.00	57.50	65.00	62.50	60.00
Fusarium oxysporum	84.44	71.11	57.77	63.33	64.44	27.77
p=0.05	2.10	4.33	6.07	5.80	5.65	9.62

Where: Value expressed in mean of triplicate, LI= *Lawsonia inermis* leaves,WSL= *Withania somnifera* leaves, DML= *Datura metel* leaves, DSL= *Datura stramonium* leaves, BRSB= *Bauhinia racemosa* stem bark and D.M-45= Dithane M-45.

Table 2. Effect of methanol extract of *Withania somnifera* leaves on seed germination and infection of seed borne pathogens.

Seed	% seed germination (h)				% infection (h)			
	24	**36**	**48**	**60**	**24**	**36**	**48**	**60**
Triticum aestivum L.								
Treated	84	100	100	100	--	--	--	--
control	100	100	100	100	--	--	--	--
Pennisetum typhoides								
Treated	51	91	92	92	--	--	--	2
control	90	92	96	96	--	--	--	12
Vigna radiate L.								
Treated	--	32	42	42	--	--	--	--
control	88	96	96	97	--	--	--	--
Cicer aeritinum L.								
Treated	--	4	4	4	2	2	5	5
control	46	94	95	98	12	16	16	17
Carthamus tinctorius								
Treated	--	93	94	96	--	--	--	5
control	92	96	96	96	--	--	--	20

Table 3. Effect of methanol leaves extract of *Withania somnifera* on radicle and plumule growth of seeds.

Seed	Average of redicle/plumule after 48 h length in (mm)		Average of redicle/plumule after 48 h length in (mm)	
	Treatment	control	Treatment	control
Triticum aestivum (Wheat)	16.6/4	39/18	16.6/4	39/18
Pennisetum typhoides (Pearl millet)	25/17.4	43.33/24	25/17.4	43.33/24
Vigna radiate (Mung bean)	9.33/NF	44.38/NF	9.33/NF	44.38/NF
Cicer aeritinum (Chickpea)	NF/ NF	26.8/ NF	NF/ NF	26.8/ NF
Vigna unguicula (Cowpea)	39.8/ NF	62/ NF	39.8/ NF	62/ NF
Carthamus tinctorius (Safflower)	18.8/ NF	25/ NF	18.8/ NF	25/ NF

NF = not formed

Table 4. Minimum inhibitory concentration (µl/ml) of *Lawsonia inermis* extract leaves against fungi.

Fungi	Control	Growth of fungi MIC (µl/ml)			
		12.5	25	50	100
Colletotrichum lindemuthianum	+	-	-	-	-
Colletotrichum capsici	+	+	+	+	+
Alternaria alternata	+	+	+	+	-
Bipolaris oryzae	+	+	+	+	-
Fusarium moniliforme	+	+	+	+	-
Curvularia lunata	+	+	+	+	-
Rhizoctonia solani	+	-	-	-	-
Microphomina phaseolina	+	+	+	+	-

Where : - = without growth, + = Growth.

Table 5. Phytochemical analysis of effective crude plant extracts.

Plant species	Part(s) used	Alkaloids	Cardiac glycosides	Flavonoids	Phenols	Saponins	Steroids	Terpenoides	Tannins
Bauhinia racemosa	Stem bark	-	-	-	-	+	-	-	-
Datura metel	Leaves	++	-	+	-	++	++	-	+
Datura stramonium	Leaves	++	-	+	-	±	+	-	-
Lawsonia inermis	Leaves	+	-	++	-	±	-	-	+++
Withania somnifera	Leaves	+	-	+	-	±	++	-	±

Where : - = Absent, ± = traces, + = low concentration, + + = medium concentration, + + + = high concentration.

Table 6. Zone of inhibition of extracts on *Candida albicans* B017.

Plant extract	Zone of inhibition (mm)	
	Yeast malt agar	Yeast malt agar + sorbitol
Lawsonia inermis	22 ± 0.32	22 ± 0.70
Datura metel	16 ± 0.71	18 ± 0.91
Withania somanifera	20 ± 0.47	24 ± 1.10
Datura stramonium	11 ± 0.40	11 ± 0.45
Bauhinia racemosa	13 ± 0.91	15 ± 0.55
Griseofulvin	--	--

Data given in mean ± S.E.M. of three replicates, -- = not found.

Table 7. Effect of methanol extract of *Lawsonia inermis* on enzyme secretion of *Aspergillus niger* and *Fusarium oxysporum.*

	Aspergillus niger		*Fusarium oxysporum*	
Enzyme	**Control**	**Treated**	**Control**	**Treated**
Amylase	+ +	+ +	+ +	++
Catalase	+ +	–	+ +	–
Dehydrogenase	+	+	+	+
Protease	+ +	+ +	+ +	+ +

Where: - = absence of enzyme, + = presence and weak activity of enzymes; + + = presence and strong activity of enzyme.

Table 8. Antifungal activity of protein and non-protein fractions of crude plant extracts on *Colletotrichum lindemuthianum* and *Bipolaris oryzae.*

Plant Extract Fraction	Mycelial growth in (mm)		% inhibition over control	
	C. lindemuthianum	*B. oryzae*	*C. lindemuthianum*	*B. oryzae*
Withania somnifera				
Protein	15 ± 0.96	30 ± 0.81	78.25	66.66
Non protein	57 ± 0.37	76 ± 0.67	17.39	15.55
Lawsonia inermis				
Protein	10 ± 0.49	9 ± 0.19	85.50	90.00
Non protein	55 ± 0.20	73 ± 0.55	20.28	18.88
Control	69 ±0.71	90 ±0.45	---	---

Data given in mean ± S.E.M. of three replicate.

Table 9. Chromatography analysis (R_f values) of effective crude plant extracts in selected thin layer chromatography solvent systems.

Solvent system	R_f values of compounds				
	BRSB	DML	DSL	LIL	WSL
Ethyl acetate / methanol / water (40:5.4:5)	0.80	0.08; 0.23, 0.79	0.11a, 0.25, 0.31d, 0.41e, 0.50, 0.77	0.63 0.68 0.80f	0.28b, 0.66h, 0.72, 0.80j.
Toluene / ethyl acetate (1:1)	0.85	0.11a, 0.20 0.31d, 0.41e, 0.29,0.76, 0.80 g, 0.86h, 0.88 i.	0.18 b, 0.28c, 0.44 f, 0.80g, 0.84, 0.87	0.15, 0.19, 0.28c, 0.40, 0.41e, 0.44f, 0.47,0.76, 0.81, 0.82, 0.88i	0.18b, 0.22, 0.32, 0.38, 0.47, 0.81k, 0.86h

The R_f values followed by letter indicates similar compounds in plant extracts. BRSB = *Bauhinia racemosa* stem bark, DML = *Datura metel* leaves, DSL = *Datura stramonium* leaves, LIL = *Lawsonia inermis* leaves, WSL = *Withania somnifera* leaves, nd = not determined.

Discussion

Antifungal activity of selected plants was reported against different fungi (Mughal *et al.,* 1996; Thirbhuvanmala and Doraisamy 2004; Pandey *et al.,* 2002, Sharma and Trivedi, 2002; Kumar, 2005). By careful observation the results found by these workers noticed, the activities of the plants are specific against particular fungi. Effect of plant extracts on percent seed germination and microbial infection was reported that extract of *Allium sativum* and *Einnicostema littorals* were controlled microbial infection while seed germination was enhanced after treatment with extracts. Though 10 % extracts inhibited seed germination, very low concentration of extracts can be used to treat seeds as a seed protectant because high concentration of extract can interfere with metabolic activity of seed (Rathore, 1995).

Extract of *L. inermis* (1%) increased the seed germination of chickpea by decreasing the infection of *F. oxysporum in vivo*. This is the first time the extract of *L. inermis* used as seed protectant *in vivo*. No reports are available in previous literature about use of this extract *in vivo* against any seeds. Therefore extract of *L. inermis* could be used as seed protectant against seed borne

pathogens like *F. oxysporum*. The MIC was varying with the fungi. Very less concentration of *L. inermis* extract were effective to control the growth of five fungi because it showed very less MIC. In some plants different types of secondary metabolite were presented. The presence of these compounds in tested plants was also reported by various workers (El-Sayed *et al.,* 1997; Ganzera *et al.,* 2003; Mikhail *et al.,* 2004). Antifungal activity exhibited by these plants may attributes to the presence of these secondary metabolites. These compounds can combat with pathogens by different mode of action.

Production of catalase enzyme in *A. niger* and *F. oxysporum was* inhibited by *L. inermis* extracts. Catalase is essential enzyme in fungi for the conversion H_2O_2 in to oxygen and water. If this enzyme is not produce then H_2O_2 will accumulate in large quantity and toxic to the cell. Effect of plant extracts on different enzyme secretion was also reported by Rathore (1995). Gupta and Bilgrami (1970) reported that decoction of bark from *Acacia arabica* and *Casuarina equisetifolia* inhibits the production and activity of cellulolytic enzyme in *Pythium aphanidermatum* and this is the line with present findings. Inhibition of enzyme by the plant extracts might be due to the loss in adaptability or it may be due to the presence of some inhibitor compound.

Protein fractions of *L. inermis* and *W. somnifera* revealed greater antifungal activity as compared to that of non-protein fractions. It confirmed that the active antifungal compounds are proteinaceous in nature or protein in both plants. Similar finding was also observed by Renukadevi (1995); Thirbhuvanmala and Doraisamy (2004) in *L. inermis* and *Prosopis juliflora* against other fungi. Further study is necessary to identify and elucidation of these compounds.

The cell walls of fungi are unique and essential for their survival. Hence whole cell of *C. albicans* screened to identify inhibitor of fungal cell wall synthesis and assembly based on osmotic support and morphological character of the cell. Damage to the cell wall component by any cell wall active agent will lyses the cell but they will continue to grow if a suitable osmo-protection like sorbitol present in the medium. None of the extract showed hazy and reduced zone of inhibition in treated plate containing sorbitol. It suggests that the mode of action of active compounds is other than cell wall. But all extract revealed zone of inhibition against *C. albicans* 3017. Anticandidal activity of these plants was reported by Kumar *et al.* (2005) and Kambizi and Afolayan, (2008). While antibiotics Greseofulvin did not revealed zone of inhibition against *C. albicans* 3017. It means the pathogen had developed resistance to this antibiotic. *C. albicans* 3017 and other species of *Candida* causing candidiasis and invades different area of the human body causing cutaneous, mucataneous are increasingly important disease throughout the world due to the

fails that they are frequently opportunistic pathogen in AIDS patient as stated by Cowan (1999).

After TLC of crude extracts, *L. inermis* showed highest number of bands. In different extracts, some similarities were observed in the chemical composition because they showed same R_f values and colour. It indicates that these plants are closely related, because particular secondary metabolites are restricted in specific taxa such as family, order, genus, etc.

The antifungal activity exhibited by the selected plants might be attributed to the presence of either single or synergetic effect of more than one compound. Out of them, *L. inermis* and *W. somnifera* were found to potent antifungal plants.

References

Abd-El-Khair, H. and Hafiz, O.M. (2001). Effect of aqueous extract of some medicinal plants in controlling the green mould disease and improvement of stored "Washington" Novel orange quality. Journal of Applied Science and Research 2(10): 664-674.

Audu, S.A., Mohammed, I. and Kaita, H.A. (2007). Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Science Journal 4(4): 75-79.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Review 12: 564-582.

Dekshit, V.R. and Desai, P.D. (2000). An Agar plate assay to detect cell active antifungal agents. Current Science 78(5) : 615-617.

Deshpande, A.R., Musaddiq, M. and Bhandange, D.C. (2004). Studies on antibacterial activity of some plant extracts. Journal of Microbial World 6(1): 45-49.

Egwaikhide, I. and Gimba, C.E. (2007). Analysis of phytochemical content and antimicrobial activity of *Plectranthus glandulosis* whole plant. Middle-East Journal of Scientific Research 2(3-4): 135-138.

El-Sayed, M.S., Ahmed, S. and Haroun, L. (1976). Influences of low irradiation doses combined with a growth regulators on growth and alkaloid content of *Datura stramonium* L. and *Datura innoxia* Miller [Drug plant in Egypt]. Egypt Journal of Pharmacology and Science 17: 321-328.

Ezzat, S.M. (2001). In vitro inhibition of *Candida albicans* growth by plant extracts and essential oil. World Journal of Microbiology and Biotechnology 17: 757-759.

Gangawane, L.V. (1990). Fungicide resistance in plant pathogens in India. Indian Phytopathology 40: 551-553.

Ganzera, M., Chaudhary, M.J. and Khan, I.A. (2003). Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia 74 (1-2): 68-76.

Gupta, C., Garg, A.P., Uniyal, R.C. and Kumari, A. (2008). Antimicrobial activity of some herbal oils against common food borne pathogens. African Journal of Microbiology Research 2:254-261.

Gupta, S. and Bilgrami, R.S. (1970). Inhibitory effect of some plant decoction on the production and activity of celluloytic enzymes of three pathogenic fungi. Academic Science of India 40: 6-8.

Kambizi, L. and Afolayan, A.J. (2008). Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrehoea*. African Journal of Biotechnology 7(1): 12-15.

Kumar, R.S., Sivakumar, T., Sunderam, R.S., Gupta, M., Mazumdar, V.K., Gomathi, P., Rajeshwar, Y., Sarawanan, S., Kumar, M.S., Murugesh, K. and Kumar, K. A. (2005). Antioxidant ad antimicrobial activity of *Bauhinia racemosa* L. stem bark. Brazilian Journal Medical and Biological Research 38: 1015-1024.

Mikhail, B.R., Badria, F.A., Maatooq, G.T. and Amer, M.M.A. (2004). Antioxidant and immunomodulatory constituents of Henna leaves. Z. Naturforsh 59: 468-476.

Mughal, M.A., Khan, T.Z. and Nasir, M.A. (1996). Antifungal properties of some plant extracts. Pakistan Journal of Phytopathology 8: 46-48.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolke, R.H. (1995). Manual of Clinical Microbiology 6thed. Washington DC, ASM.

Pandey, M.K., Singh, A.K. and Singh, R.S. (2002). Mycotoxic potential of some higher plants. Plant Disease Research 17(1): 51-56.

Rathore, O.S. (1995). Antimicrobial activity of some medicinal plants. Ph. D. thesis Dr. Babasaheb Ambedkar University Aurangabad, India.

Renukadevi, P. (1995). Management of chili die back and fruit rot disease with plant extracts and fungicide. M. Sc. (Agri.) thesis. Department of plant pathology, Tamil Nadu Agriculture University Coimbatore, India. pp. 111.

Schmitz, H. (1930). Food poisoned technique. Industrial Engineering Chemical Analyst Education p.361-363.

Sharma, N. and Trivedi, P.C. (2002). Screening of leaf extracts of some plants for their Nematocidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. Asian Journal of Experimental Science 16 (1-2): 21-28.

Thirbhuvanamala, G. and Doraisamy, S. (2004). Effect of plant extracts against *Diplocarpon rosae* the black spot pathogen of rose. Journal of Microbial World 6 (1): 67-71.

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