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Fungicidal Effect of Phytoconstituents of Medicinal Plants

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ABSTRACT

The antimicrobial chemicals create health hazards for all organisms and the remedy to the health hazards is switching to bio-ingredients such as phytoconstituents of the medical plants as antimicrobial agents. This research analyses the fungicidal effect of phytoconstituents of medicinal plants. Leaves of *Withania sominifera, Azadirachta indica, Solanum virginianum,* and fruits and rhizome of *Solanum virginianum* and *Alocasia odora* were used to extract the phytoconstituents for the inhibition of fungi, *Cladosporium sp., Guignardia citricarpa, Alternaria alternate, Penicillium sp., Rhizopus stolonifer*. The morphogenesis of growing pathogen in potato dextrose broth was identified and measured using spectrophotometer. 50% acetone is a suitable solvent for the extraction of phytoconstituents in this inhibition study. Fungal *sp*. can be inhibited by lowest concentration of solvent (5mg/ml or 10mg/ml), 50% acetone. Some species of fungi can be inhibited strictly with the phytoconstitutents of particular part of the plants only. Phytoconstituents of fruits of S*olanum virginianum* in 50% acetone solvent at 5mg/ml concentration is effective fungicide for the inhibition of *Alternaria* sp. *Guignardia citricarpa*, *Penicilium sp., Rhizopus* sp. and *Cladosporium sp.*

KEYWORDS: Phytoconstituents, Antimicrobial, fungicide, Medicinal plants, Inhibition

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1. INTRODUCTION

Antibiotic resistance is a serious problem in many countries, both in developed and developing countries due to increased inappropriate use, ineffectiveness and human mortality[1, 2]. Alternative to insecticides, pesticides, bactericides, fungicides etc. may be the extracts of medicinal plants which have antimicrobial activity[3, 4]. Methanolic extracts from *Peganum harmala* (Zygophyllaceae), *Ajuga iva* (Labiateae), *Aristolochia baetica* (Aristolochiaceae) and *Raphanus raphanistrum* (Brassicaceae) were used to control pest, *Tribolium castaneum* and found effective[5]. Antimicrobial activity of ethanolic extracts of *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales* and *Thymus vulgaris* against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using at concentration of 10 mg/ml were found effective respectively while extract of *Cuminum cyminum* was only effective against *Staphylococcus aureus*[6]. The mother tincture extract of *Myroxylon balsamum* has been used for antifungal activity, for the inhibition of phytopathogenic fungi[7]. The antifungal and antibacterial activity can be observed in many fruits bearing commonly used trees such as *Tamarindus indica, Acacia nilotica, Mangifera indica* etc.[8]. The aqueous, ethanolic and ethyl acetate extracts of neem leaves, *Azadirachta indica* on human pathogens, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Microsporum gypseum* in vitro at different concentrations, 5%, 10%, 15% and 20% yielded good result[9]. The antifungal potential may be due to the presence of some polar constituents such as glycoside, saponins, tannins etc. which may be present in the extract[10]. This research is to analyze the percentage of extraction of phytoconstituents from different medicinal plants using different solvents and their effect as fungicides for the treatment of fungal pathogens dose-wise.

2. MATERIALS AND METHODS

Phytoconstituents

Leaves of *Withania sominifera, Azadirachta indica* [11], *Solanum virginianum*, and fruits and rhizome of *Solanum virginianum* and *Alocasia odora* respectively were collected from Jaipur National University campus and dried. Leaves, fruits and rhizome were ground and made fine powder. The powder was stored for extraction. One-gram powder each was dissolved in 50% acetone, 80% acetone and water. The mixture could stay for two hours with often shaking and filtered using filter paper. The filtrate was evaporated, and the dry phyto-constituent was weighed. The percentage of extraction was calculated using formula;

Percentage of extraction = weight of extracted dry powder /dry weight of sample powder

The dried extracts were dissolved in their respective solvents, diluted to make uniform concentration and stored at -4-degree Celsius fridge for further use. The phytoconstituents were extracted from five different medicinal plant products, table 1 and the extraction was done by maceration method using acetone and water as per the selection of solvents. To analyze the effect of the phytoconstituents, the extraction was done using water, 50% acetone and 80% acetone. To find the dose-wise effect of the phytoconstituents, the phytoconstituents were diluted to get 5mg/ml, 10mg/ml and 20 mg/ml. Table 1 shows all the details of the plants used for phytoconstituents.

Table 1 Name of the plants and the plant parts used in the study study

S.No	Name of the plant	Family of plant	Parts used
1	Withania somenifera	Solanaceae	Leaves
2	Azadirachta indica	Meliaceae	Leaves
3	Solanum virginianum	Solanaceae	Leaves
4	Solanum virginianum	Solanaceae	Fruit
5	Alocasia odora	Araceae	Rhizome

Pathogen spore suspension

The common disease-causing fungi were isolated from plants causing respective diseases, table 2 and a small infected piece of leave was transferred into culture medium, potato dextrose agar. After seven days, the mycelium and spores were identified under microscopic observation. The spores were extracted from the mycelium colony and filtered using filter cloths and centrifuged the filtrate at 5000 rpm for 10 minutes. The spores were washed with water and re-centrifuged twice. Spores were counted using hemo-cytometer for making spore suspension, spores/ml and stored in 20% glycerol at -4 degree Celsius. Number of spores per ml = average x division factor x10^4)

The common pathogens used are given in the table 2

Culture medium

PDA was used for the culture of the pathogen. For the preparation100 ml of PDA, 3.9 gm of PDA was added in 50 ml of water, then 0.05 gm of agar was added, then volume make up done by adding 50 ml more water in it. Boil it for making agar soluble and autoclave it. PDB was used in inhibition study. PDB, potato dextrose broth medium was melted using sterile water and autoclaved along with Petri discs, water, test tubes etc.

Table 2 Names of pathogens studied and their details

	Name of the Fungi	Name of the Phylum	Name of the Class	Name of family	Name of the host	Name of the disease
1	*Cladosporium sp.*	Ascomycota	Dothideomycetes	Davidiellaceae	Prunus persica	Scab on peach fruits
2	*Guignardia citricarpa*	Ascomycota	Dothideomycetes	Botryosphaeriaceae	Citrus limon	Citrus black spot
3	*Alternaria alternate*	Ascomycota	Dothideomycetes	Pleosporaceae	Solanum melongena	Leaf spot
4	*Penicillium sp.*	Ascomycota	Eurotiomycetes	Trichomaceae	Citrus limon	Green mold decay
5	*Rhizopus stolonifer*	Zygomycota	Zygomycetes	Mucoraceae	Carica papaya	Storage rot

Inhibition study

The inhibition study by all pathogens were conducted as follows: seven ml of PDB, 200 micro liters of spores of pathogen and 200 micro liters of 50% acetone in one test tube, in other test tube 80% acetone and all as above and the third test tube water and all as above and these were taken as controls and with phytoconstitutents as experiments. One test was taken with seven ml of PDB, 200 micro liters of spores of pathogen only for reference to know the growth of microbes. This is for one plant and one pathogen. Like five pathogen and five plant's phytoconstituents were taken in test tubes with well label. After taking absorbance at 595 nm at 0-hours, the experiment starts by keeping all the test tubes in an incubator at 45 degree Celsius. The absorbance was taken after every one hour and the results were recorded for calculating the total inhibition of phytoconstituents alone.

3. RESULTS

80% and above inhibition of each fungus is generated after deleting the percentage of inhibition below 80%, table 3

It is clear from the table that Penicillium is inhibited by phytoconstituents of all concentrations of medicinal plants and phytoconstituents of solvent for the extraction by 80% acetone inhibit all species of selected fungi. Therefore, a new table, table 4 is created after deleting the clear results and filling actual values of "*" to find the significant levels for effective concentration of inhibition.

Table 3 80% and above Inhibition of the selected fungi by different selected phytoconstituents

Phytoconstituents		Inhibition of fungi				
Solvent concentration for extraction	**Dose-wise concentration of Phytoconstituents**	**Inhibition of *Cladosporium sp.***	**Inhibition of *Guignardia citricarpa***	**Inhibition of *Alternaria alternata***	**Inhibition of *Penicilium sp.***	**Inhibition of *Rhizopus stolonifer***
Rhizome (*Alocasia odora*)						
50% Acetone as solvent	5mg/ml	*	100%	96%	95%	86%
	10mg/ml	89%	89%	88%	94%	89%
	20mg/ml	82%	97%	79%	94%	98%
80% Acetone as solvent	5mg/ml	100%	100%	89%	93%	92%
	10mg/ml	100%	100%	94%	91%	93%
	20mg/ml	93%	100%	85%	93%	95%
Water as solvent	5mg/ml	100%	94%	*	97%	*
	10mg/ml	100%	97%	*	95%	*
	20mg/ml	100%	93%	*	93%	*
Seed (S*olanum virginianum*)						
50% Acetone as solvent	5mg/ml	*	*	91%	*	91%
	10mg/ml	99%	*	84%	91%	96%
	20mg/ml	*	86%	85%	99%	96%
80% Acetone as solvent	5mg/ml	100%	100%	92%	86%	92%
	10mg/ml	100%	100%	94%	97%	94%
	20mg/ml	*	100%	92%	89%	95%
Water as solvent	5mg/ml	100%	94%	*	97%	*
	10mg/ml	100%	96%	*	96%	*
	20mg/ml	100%	94%	*	95%	*
Fruit (*Solanum virginianum)*						
50% Acetone as solvent	5mg/ml	91%	*	98%	*	82%
	10mg/ml	*	*	83%	*	87%
	20mg/ml	90%	*	94%	87%	100%
80% Acetone as solvent	5mg/ml	100%	100%	88%	92%	93%
	10mg/ml	100%	100%	91%	95%	94%
	20mg/ml	100%	97%	86%	100%	96%
Water as solvent	5mg/ml	100%	93%	*	82%	*
	10mg/ml	100%	87%	79%	90%	*
	20mg/ml	100%	85%	*	97%	*
Leaves (*Withania somenifera*)						
50% Acetone as solvent	5mg/ml	84%	*	*	86%	97%
	10mg/ml	94%	*	*	90%	95%
	20mg/ml	85%	*	*	92%	96%
80% Acetone as solvent	5mg/ml	100%	100%	84%	98%	89%
	10mg/ml	*	100%	93%	96%	89%
	20mg/ml	*	100%	90%	92%	94%
Water as solvent	5mg/ml	100%	91%	*	91%	*
	10mg/ml	100%	88%	*	93%	*
	20mg/ml	100%	90%	*	96%	*
Leaves (Azadirachta indica)						
50% Acetone as solvent	5mg/ml	100%	83%	100%	83%	100%
	10mg/ml	*	81%	100%	81%	96%
	20mg/ml	79%	90%	100%	89%	97%
80% Acetone as solvent	5mg/ml	100%	100%	89%	94%	94%
	10mg/ml	100%	100%	90%	95%	92%
	20mg/ml	100%	100%	87%	96%	94%
Water as solvent	5mg/ml	98%	85%	*	93%	*
	10mg/ml	100%	90%	*	95%	*
	20mg/ml	100%	*	*	97%	*

* indicates the inhibition by less than 80%.

Table 4 80% and above Inhibition of the selected fungi by different selected phytoconstituents

Phytoconstituents		Inhibition of fungi			
Solvent concentration for extraction	Dose-wise concentration of Phytoconstituents	Inhibition of *Cladosporium sp.*	Inhibition of *Guignardia citricarpa*	Inhibition of *Alternaria alternata*	Inhibition of *Rhizopus stolonifer*
Rhizome (*Alocasia odora*)					
50% Acetone as solvent	5mg/ml	67%	100%	96%	86%
	10mg/ml	89%	89%	88%	89%
	20mg/ml	82%	97%	79%	98%
Water as solvent	5mg/ml	100%	94%	64%	50%
	10mg/ml	100%	97%	64%	50%
	20mg/ml	100%	93%	60%	50%
Seed (*Solanum virginianum*)					
50% Acetone as solvent	5mg/ml	55%	76%	91%	91%
	10mg/ml	99%	78%	84%	96%
	20mg/ml	46%	86%	85%	96%
Water as solvent	5mg/ml	100%	94%	71%	50%
	10mg/ml	100%	96%	77%	50%
	20mg/ml	100%	94%	65%	50%
Fruit (*Solanum virginianum)*					
50% Acetone as solvent	5mg/ml	91%	69%	98%	82%
	10mg/ml	36%	71%	83%	87%
	20mg/ml	90%	60%	94%	100%
Water as solvent	5mg/ml	100%	93%	71%	50%
	10mg/ml	100%	87%	79%	50%
	20mg/ml	100%	85%	36%	50%
Leaves (*Withania somenifera*)					
50% Acetone as solvent	5mg/ml	84%	51%	71%	97%
	10mg/ml	94%	39%	78%	95%
	20mg/ml	85%	66%	73%	96%
Water as solvent	5mg/ml	100%	91%	57%	50%
	10mg/ml	100%	88%	56%	50%
	20mg/ml	100%	90%	72%	50%
Leaves (Azadirachta indica)					
50% Acetone as solvent	5mg/ml	100%	83%	100%	100%
	10mg/ml	74%	81%	100%	96%
	20mg/ml	79%	90%	100%	97%
Water as solvent	5mg/ml	98%	85%	77%	50%
	10mg/ml	100%	90%	42%	50%
	20mg/ml	100%	78%	41%	50%

For finding the significant level of inhibition of fungi by phytoconstituents and to calculate the correct concentration of phytoconstituents for inhibition, SPSS (Statistical Package for Social Sciences) software was used. Since it is a table of many rows and columns, univariate module for each fungus was used from the SPSS software, tables 5, 6, 7 & 8.

Table 5 Parameter Estimates for *Cladosporium sp.*

Parameter	B	Std. Error	t	Sig.	95% Confidence Lower Bound	95% Confidence Upper Bound	Observed Power[a]
[Phytocons=10mg]	100.233	9.749	10.282	0.000	79.752	120.715	1.000
[Phytocons=20mg]	99.233	9.749	10.179	0.000	78.752	119.715	1.000
[Phytocons=5mg]	100.533	9.749	10.312	0.000	80.052	121.015	1.000
[Plants=Aloca50]	-20.667	12.586	-1.642	0.118	-47.108	5.775	0.343
[Plants=AlocaWat]	-9.255E-14	12.586	0.000	1.000	-26.441	26.441	0.050
[Plants=Azadi50]	-15.667	12.586	-1.245	0.229	-42.108	10.775	0.218
[Plants=AzadiWa]	-.667	12.586	-.053	0.958	-27.108	25.775	0.050
[Plants=SolFru50]	-27.667	12.586	-2.198	0.041	-54.108	-1.225	0.548
[Plants=SolFruWa]	-9.255E-14	12.586	0.000	1.000	-26.441	26.441	0.050
[Plants=SolSee50]	-33.333	12.586	-2.649	0.016	-59.775	-6.892	0.707
[Plants=SolSeeWa]	-9.049E-14	12.586	0.000	1.000	-26.441	26.441	0.050
[Plants=With50]	-12.333	12.586	-.980	0.340	-38.775	14.108	0.153
[Plants=WithWa]	0[b]	.	.	.	.	.	.

Computed using alpha = .05, b. This parameter is set to zero because it is redundant. Phytocons = Phytoconstituents, Aloca50 = Rhizome of *Alocasia odora* in 50% acetone solvent, AlocaWat = Rhizome of *Alocasia odora in* water solvent, Azadi50 = *Azadirachta indica* in 50% acetone solvent, AzadiWa = *Azadirachta indica* in water solvent, SolFru50 = Fruit of S*olanum virginianum* in 50% acetone solvent, SolFruWa = Fruit of S*olanum virginianum* in water solvent, SolSee50 =Seed of Solanum virginianum in 50% acetone solvent, SolSeeWa = Seed of Solanum virginianum in water solvent

Table 6 Parameter Estimates for *Guignardia citricarpa*

Parameter	B	Std. Error	t	Sig.	95% Confidence		Observed Power[a]
					Lower	Upper Bound	
[Phytocons=10mg]	88.23	3.922	22.49	.000	79.99	96.47	1.000
[Phytocons=20mg]	90.533	3.922	23.086	.000	82.294	98.772	1.000
[Phytocons=5mg]	90.233	3.922	23.009	.000	81.994	98.472	1.000
[Plants=Aloca50]	5.667	5.063	1.119	.278	-4.970	16.303	.185
[Plants=AlocaWat]	5.000	5.063	.988	.336	-5.637	15.637	.155
[Plants=Azadi50]	-5.000	5.063	-.988	.336	-15.637	5.637	.155
[Plants=AzadiWa]	-5.333	5.063	-1.053	.306	-15.970	5.303	.170
[Plants=SolFru50]	-23.000	5.063	-4.543	.000	-33.637	-12.363	.990
[Plants=SolFruWa]	-1.333	5.063	-.263	.795	-11.970	9.303	.057
[Plants=SolSee50]	-9.667	5.063	-1.909	.072	-20.303	.970	.439
[Plants=SolSeeWa]	5.000	5.063	.988	.336	-5.637	15.637	.155
[Plants=With50]	-37.667	5.063	-7.440	.000	-48.303	-27.030	1.000
[Plants=WithWa]	0[b]	.	.	.	.	.	.

Computed using alpha = .05, b. This parameter is set to zero because it is redundant. Phytocons = Phytoconstituents, Aloca50 = Rhizome of *Alocasia odora* in 50% acetone solvent, AlocaWat = Rhizome of *Alocasia odora in* water solvent, Azadi50 = *Azadirachta indica* in 50% acetone solvent, AzadiWa = *Azadirachta indica* in water solvent, SolFru50 = Fruit of S*olanum virginianum* in 50% acetone solvent, SolFruWa = Fruit of S*olanum virginianum* in water solvent, SolSee50 =Seed of Solanum virginianum in 50% acetone solvent, SolSeeWa = Seed of Solanum virginianum in water solvent.

Table 7 Parameter Estimates for *Alternaria alternata*

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Observed Power[a]
					Lower Bound	Upper Bound	
[Phytocons=10mg]	61.700	6.726	9.173	.000	47.569	75.831	1.000
[Phytocons=20mg]	57.100	6.726	8.489	.000	42.969	71.231	1.000
[Phytocons=5mg]	66.200	6.726	9.842	.000	52.069	80.331	1.000
[Plants=Aloca50]	26.000	8.684	2.994	.008	7.756	44.244	.808
[Plants=AlocaWat]	1.000	8.684	.115	.910	-17.244	19.244	.051
[Plants=Azadi50]	38.333	8.684	4.414	.000	20.090	56.577	.986
[Plants=AzadiWa]	-8.333	8.684	-.960	.350	-26.577	9.910	.149
[Plants=SolFru50]	30.000	8.684	3.455	.003	11.756	48.244	.904
[Plants=SolFruWa]	.333	8.684	.038	.970	-17.910	18.577	.050
[Plants=SolSee50]	25.000	8.684	2.879	.010	6.756	43.244	.777
[Plants=SolSeeWa]	9.333	8.684	1.075	.297	-8.910	27.577	.175
[Plants=With50]	12.333	8.684	1.420	.173	-5.910	30.577	.270
[Plants=WithWa]	0[b]	.	.	.	.	.	.

Computed using alpha = .05, b. This parameter is set to zero because it is redundant. Phytocons = Phytoconstituents, Aloca50 = Rhizome of *Alocasia odora* in 50% acetone solvent, AlocaWat = Rhizome of *Alocasia odora in* water solvent, Azadi50 = *Azadirachta indica* in 50% acetone solvent, AzadiWa = *Azadirachta indica* in water solvent, SolFru50 = Fruit of S*olanum virginianum* in 50% acetone solvent, SolFruWa = Fruit of S*olanum virginianum* in water solvent, SolSee50 =Seed of Solanum virginianum in 50% acetone solvent, SolSeeWa = Seed of Solanum virginianum in water solvent.

Table 8 Parameter Estimates for *Rhizopus stolonifer*

Parameter	B	Std.	t	Sig.	95% Confidence Interval		Observed Power[a]
					Lower Bound	Upper Bound	
[Phytocons=10mg]	49.433	2.237	22.101	.000	44.734	54.133	1.000
[Phytocons=20mg]	51.833	2.237	23.174	.000	47.134	56.533	1.000
[Phytocons=5mg]	48.733	2.237	21.788	.000	44.034	53.433	1.000
[Plants=Aloca50]	41.000	2.888	14.199	.000	34.933	47.067	1.000
[Plants=AlocaWat]	-5.235E-14	2.888	.000	1.000	-6.067	6.067	.050
[Plants=Azadi50]	47.667	2.888	16.507	.000	41.600	53.733	1.000
[Plants=AzadiWa]	-5.290E-14	2.888	.000	1.000	-6.067	6.067	.050
[Plants=SolFru50]	39.667	2.888	13.737	.000	33.600	45.733	1.000
[Plants=SolFruWa]	-4.879E-14	2.888	.000	1.000	-6.067	6.067	.050
[Plants=SolSee50]	44.333	2.888	15.353	.000	38.267	50.400	1.000
[Plants=SolSeeWa]	-5.290E-14	2.888	.000	1.000	-6.067	6.067	.050
[Plants=With50]	46.000	2.888	15.930	.000	39.933	52.067	1.000
[Plants=WithWa]	0[b]	.	.	.	.	.	.

Computed using alpha = .05, b. This parameter is set to zero because it is redundant. Phytocons = Phytoconstituents, Aloca50 = Rhizome of *Alocasia odora* in 50% acetone solvent, AlocaWat = Rhizome of *Alocasia odora in* water solvent, Azadi50 = *Azadirachta indica* in 50% acetone solvent, AzadiWa = *Azadirachta indica* in water solvent, SolFru50 = Fruit of S*olanum virginianum* in 50% acetone solvent, SolFruWa = Fruit of S*olanum virginianum* in water solvent, SolSee50 =Seed of Solanum virginianum in 50% acetone solvent, SolSeeWa = Seed of Solanum virginianum in water solvent

Tables 5 – 8 show that 5mg/ml, 10mg/ml and 20mg/ml (P-value 0.000 and Power 1.00) are effective in inhibiting all selected fungal species on study (Statistically significant) however, the plant and parts used for extracting the phytoconstituent for inhibition vary. *Cladosporium sp*. can be inhibited effectively by fruit and seed of S*olanum virginianum* in 50% acetone as solvent in both cases (P-values 0.041 and 0.016, statistically significant), *Guignardia citricarpa.* can be inhibited by using both, *Withania somenifera* and fruit of S*olanum virginianum*, both in 50% acetone as solvent which gives statistically significant result (P-value 0.000), *Alternaria alternata* can be inhibited by Rhizome of *Alocasia odora*, fruit and seed of S*olanum virginianum* and *Azadirachta indica* in 50%

acetone as solvent in all four cases ((P-value 0.008, 0.003, 0.010 and 0.000 respectively) and *Rhizopus stolonifer* can be inhibited by all five medicinal plants and parts in 50% acetone as solvent in all cases (P-value 0.000 for all cases, statistically significant).

This result is in agreement with the minimum concentration of the phytoconstituent as 5 mg/ml for the effective inhibition of the pathogen.

DISCUSSION

The inhibition of fungi by phytoconstituents obtained from the spectrophotometric readings are analyzed which clearly showed that in some cases that the 2 hours of inhibition of fungal species is more than the 4 hours of inhibition and the inhibition rate is more than 100% (There are possibilities of getting inhibition more than 100%, due to the high growth in the control, less level of phytoconstituents), data not attached. Therefore, the tabulation for inhibition was taken from both the columns (2 hours and 4 hours) and chosen the highest values.

Penicillium sp can be inhibited by any concentration of the phytoconstituents

Concentrations of the phytoconstituents, 5mg/ml, 10mg/ml and 20mg/ml are obtained from each parts of the medicinal plants to find the minimum concentration of the phytoconstituents required for the inhibition of the fungi and it is evident from the table 3 that *Penicillium sp*. are inhibited[12] by all concentrations of phytoconstituents however, inhibition of other fungi are not clear from the table 3 and hence significant level of inhibition was found by using SPSS software. The analysis using software gives clarity of statistical significant level for using all concentrations of phytoconstituents for the inhibition of the fungi selected on study (P-value 0.000 for all concentrations of phytoconstituents and Power 1.00, which is very much required).

Phytoconstituents extracted using 80% acetone is highly effective in inhibiting all types of pathogen

Phytoconstituents extracted using 80% acetone as solvent is highly effective in inhibiting all the fungal species on study (more than 80% of inhibition). This may be due to the over concentration of extraction of additional phytoconstituents and their effect in their inhibition. The remaining concentration of the solvents, 50% acetone and water used in extraction did not give clear cut inhibition of the selected fungi on study from the table 3. Therefore, SPSS software was used to find the significant level.

5mg/ml is the minimum concentration of solvent required for the inhibition of growth of pathogens

The better minimum concentration of solvent for extraction can be 50% acetone, obtained from the tables 5-8. *Penicillium sp*., as in the tables 3 is inhibited by all concentrations of phytoconstituents of all selected medicinal plants hence, the species can be inhibited easily by minimum concentration of 5mg/ml in an inexpensive extraction of using water as solvent[13].

Inhibition of pathogen is specific to specific parts of medicinal plants, specific constitutents

Cladosporium sp. can be inhibited effectively by using fruit and seed of S*olanum virginianum* in 50% acetone as solvent[14], table 5, *Guignardia citricarpa.* can be inhibited by using both, *Withania somenifera* leaves[15, 16] and fruit of S*olanum virginianum*, both in 50% acetone as solvent table 6, *Alternaria alternata*[17, 18] can be inhibited by Rhizome of *Alocasia odora*[19], fruit and seed of S*olanum virginianum* and *Azadirachta indica*[20, 21] in 50% acetone as solvent table 7 and *Rhizopus stolonifer* can be inhibited by all five medicinal plants and parts in 50% acetone as solvent in all cases, table 8.

In agreement with this study, Sailaja[22] demonstrated the potent antifungal activity of methanolic stem extracts from *W. somnifera against Alternaria alternata, Curvularia lunata,* and *Candida albicans*. In another study, *W. somnifera* was found to be very effective against human *Apergillus* infections[4]. Girish *et al*.,2006 have demonstrated that *W. somnifera* root aqueous extract contains a glycoprotein with fungistatic effect toward three phytopathogenic fungi[15]. *Azadirachta indica* leaves possess good anti-fungal activity, confirming the use of this plant in primary health care[23]. Ethyl acetate fraction of the leaves of *A. indica* has antifungal activity because of the presence of three tetracyclic triterpenoids bioactive compound[24.] *Alocasia odora* has been identified that Alocasin, may be the antifungal present in the rhizomes of Alocasia macrorrhiza as isolated by Wang *et al*, 2003,[19] which may justify the same antifungal activity in Alocasia odora. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

5. SUMMARY AND CONCLUSION

50% of acetone is suitable solvent for the extraction of phytoconstituents. Fungual sps. can be inhibited by lowest concentration of solvent (5mg/ml or 10mg/ml) in 50% acetone solvent. Some

species of fungi can be inhibited strictly with the phytoconstitutents of particular part of the plants only. Inhibition of microbes by phytoconsitutients is environment friendly. In conclusion, Phytoconstituents of fruits of S*olanum virginianum* in 50% acetone solvent at 5mg/ml concentration is effective fungicide for the inhibition of *Alternaria* sp. *Guignardia citricarpa*, *Penicilium sp.*, *Rhizopus* sp. and *Cladosporium sp.*

List of abbreviations: Not Applicable, all abbreviations are expanded in the text itself.

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