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Preliminary Screening of *Sapindus mukorossi* Extracts from Different Sources against Forest Fungi

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ABSTRACT

Background: Secondary metabolites of plant have shown antimicrobial activity. Saponins, from pericarp of *S. mukorossi* have been reported to possess antimicrobial efficacy against variety of bacteria and fungi. The present study entails to asses this efficacy against different forest fungi.

Methods: Seeds of *S. mukorossi*, a medicinal plant of family Sapindaceae, collected from different sources viz. Dehradun and Gyarahdevi (Uttarakhand) and Nainatikker (Himachal Pradesh) were tested for their antifungal properties on the basis of IC_{50}. Chloroform and methanol extract of fruit pericarp from three sources of soap nut were assayed against eight forest fungi using poisoned food technique. Both the extracts were screened at four concentrations of 0.5%, 1.0%, 1.5% and 2%.

Results: In our screening, we have found that chloroform extract of all sources registered IC_{50} at all concentrations against *Phoma* sp. and *P. dalbergiae* whereas, methanol extract of all the three sources attained IC_{50} at all concentrations against *Phoma* sp., *P. dalbergiae, R. solani* and *T. piluliferum.* IC_{50} was not achieved against *A. alternata* and *F. oxysporum* at any concentration of the sources in both the extracts.

Conclusion: It can be concluded from the present study that though saponins of soap nut tree have many useful biological activities but chloroform and methanol extracts do not contain comparable biological activity against common forest fungi.

Key-words: Antifungal activity, Forest fungi, IC_{50}, Pericarp, Poisoned food technique, Seed sources

INTRODUCTION

The Plant Kingdom has been the safeguard for the humans throughout recorded history. The importance of medicinal plants is gaining attention because of this resumption of interest. However, this is occurring while natural habitats in countries of origin are being lost. It is determined that there are about 2,500,000 species of higher plants and the majority of these have remained un-examined in detail for their pharmacological activities [1]. Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has given us a very rich botanical wealth with diverse varieties of plants growing in different parts of the country [2].

Higher plants harbor numerous compounds which provide resistance to pathogenic organisms. Towards solving the antimicrobial resistance issue, drivers of resistance and possible solutions have been listed for future approaches. Discovery and development of new antimicrobial agents that have clinical significant importance from natural resources could be one of the effective approaches. It is important to discover new antimicrobial agents in order to replace currently available antimicrobials [3].

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During the course of evolution, the selection pressure caused by pathogens has probably been highly acute and followed the vast chemical diversity in plants. Secondary compounds from plants are guaranteed to have biological activity, protecting the plant from pathogens. Information of the pests to which the secondary compounds produced in the plants are resistant may provide useful leads in predicting which pests may be controlled by compounds from a particular plant species. This approach has conducted the discovery of different botanical pesticides [4].

Sapindus mukorossi Gaerten (Family: Sapindaceae), a medicinal plant, commonly known as *Ritha* or *areetha* was found throughout India. The fruit contains saponins (10-11.5%), sugars (10%) and mucilages [5]. The fruits of the plant are valued for saponins present in plants, which consists of 56.5% of the drupe. Saponins have been isolated from the pericarp. Saponins exhibit potent antifungal property and are often present in relatively high levels in healthy plants; therefore these molecules have been implicated as determinants of plants resistance to fungal attack. Fungi that invade saponin containing plants must have strategies for protecting themselves from host saponins. For many fungi, saponin resistance may be a prerequisite for successful infection [6].

It was reported that saponins extracted from the fruit pericarp of *S. mukorossi* had bactericidal/bacteriostatic property against *L. acidophilus* [7]. It was also found that ethanol and chloroform extracts of *S. mukorossi* showed antibacterial activity against *Helicobacterium pylori* [8]. Pericarps of *S. mukorossi* exhibited potent antimicrobial activities on dermatophytes, *Epidermophyton floccosum, Trichophyton mentagrophytes, T. rubrum, Sabouraudites canis* and *C. albicans* [9].

The present study is based upon the provenance study of *S. mukorossi* collected from three different sources i.e. Forest Research Institute, Dehradun and Gyarahdevi, Pithoragarh, Uttarakhand and Nainatikker, Sirmaur, Himachal Pradesh. The main objective of this study was to test two extracts (Chloroform and methanol) of *S. mukorossi* from different sources for antifungal properties on the basis of IC_{50}. Most importantly, antifungal activity of chloroform and methanol extracts of fruit pericarp was reported for the first time in this paper.

MATERIALS AND METHODS

Preparation of fruit pericarp extracts- The present study was carried out in October, 2011 in Forest Pathology Division, Forest Research Institute, Dehradun, Uttarakhand, India.

For the preparation of extracts, pericarp was separated from seeds. The collected pericarp was spread on blotter paper sheet and air dried in shade. The dried pericarp was then cut into pieces and extracted successively with the solvents of increasing polarity viz. chloroform and methanol in a Soxhlet apparatus. These extracts were concentrated on a water bath to small volumes [10]. The yield of extracts was determined on moisture free basis (Table 1). Eight forest fungi were selected for bioassay viz. *Alternaria alternata*, *Colletotrichum gloeosporioides, Phoma* sp., *Phomopsis dalbergiae*, *Ganoderma lucidum, Fusarium oxysporum, Rhizoctonia solani,* and *Trichoderma piluliferum*. The rationale for short-listing these fungi is ecological, host specificity, plant part infectivity, wider presence, economic and loss, etc.

Table 1: Yield of extracts (%) from different sources of *S. mukorossi*

Extract	Source/Yield (%)		
	Dehradun	Gyarahdevi	Nainatikker
Chloroform	2.5	3.1	5.6
Methanol	60.0	68.7	61.8

The extracts of *S. mukorossi* were tested for their toxicity against fungal pathogens by the Poisoned Food Technique [11] on the basis of Inhibitory Concentration (IC_{50}).

Inhibitory Concentration (IC_{50})-The toxicity of extracts (chloroform and methanol) of the pericarp of *S. mukorossi* from different sources was determined against eight test fungi. A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 7 days) at the optimum temperature (25°±1°C) for growth. Chloroform extract from all three sources was dissolved in acetone solvent to prepare the concentration (%). Methanol extract from all three sources was dissolved in sterilized distilled water to make concentration (%). The solvents used for dissolving were taken on the basis of polarity.

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PDA supplemented with different plant extracts at four concentrations (0.5, 1.0, 1.5 & 2.0 %).

After solidification, small disc (0.7 cm dia) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down at the center of a Petri-dish. Suitable checks were maintained where the culture discs were grown under the same conditions on PDA without extract. Solvent checks (a solvent which was used for dissolving extract i.e. acetone) were maintained to check out the inhibitory effect of solvent on fungi. Petri plates were incubated at 25°±1°C. The radial growth of fungus colony was measured after every twenty-four hours till the fungus in the control plate completely occupied it. Three replications were maintained. The antifungal activity was evaluated by measuring the relative growth of fungus in treatment vis-a-vis control.

The percent growth inhibition over control was worked out using the formula of vincent [12].

$$I (\%) = C - T/C \times 100$$

Where,

I (%) = Growth Inhibition (%)

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

RESULTS

Chloroform extract- In this study, Table 2 showed that irrespective of concentrations, maximum and significantly more antifungal activity was found in pericarp of seeds collected from Dehradun (37.8%) against *A. alternata* and minimum inhibition was recorded in Nainatikker (31%). The growth suppression of *A. alternata* significantly increased with changing concentrations of chloroform extract, for example, highest suppression was in case of 2% (44.5%) and lowest at 0.5% concentration (41%) of chloroform extract, irrespective of seed sources.

Interactions between source and concentration (SxC) showed that Dehradun source was significantly better in terms of suppressing the growth of *A. alternata* than other two seed sources at all concentrations of chloroform extract (Table2). Barring Nainatikker source, there was overlapping growth suppression of the pathogen in relation to concentration. For example, the growth inhibition of the fungus remained at par between the concentrations of 1.5 and 2% in both Dehradun (48.5 & 49.3 % respectively) and Gyarahdevi (42.8 & 43.6% respectively) sources.

The mycelial growth of *C. gloeosporioides* was suppressed maximum and significantly more in Dehradun source 40% shown in Table 2. However, Gyarahdevi (33%) and Nainatikker (33.3%) were at par, irrespective of concentrations. There was a significant increase of fungal growth inhibition over concentrations, irrespective of seed sources.

Following interactions between source and concentration (SxC), it was observed that Dehradun source was significantly superior over the other two sources at all concentrations barring 0.5% (Table 2). Similarly, growth inhibition of *C. gloeosporioides* was significantly more at higher concentration of 1.5 (44.5%) and 2.0 (52.7%) in Gyarahdevi, however, it was significantly better at the lower concentration of 0.5 (37%) and 1.0 (40%) concentration in Nainatikker. There was a significant increase in growth suppression of the pathogen over concentration in all the sources.

The growth inhibition of *Phoma* sp. was highest and significantly more in Nainatikker source (72.0%) on the perusal of Table 2 and Fig. 1. However, minimum inhibition was reported in Dehradun (50.9%) irrespective of concentrations. There was a significant increase of fungal growth inhibition over concentrations, irrespective of seed sources. Interactions between source and concentration (SxC) revealed that 100% inhibition of growth was exhibited by Nainatikker source starting from lowest concentration of 0.5% (Table 2 and Fig. 1). In the rest of the sources, there was linearity of significant fungal growth suppression over concentrations.

Irrespective of concentrations, Nainatikker (54.3%) had highest reduction of growth of *P. dalbergiae* whereas Dehradun (52.7%) and Gyarahdevi (52.4%) suppressed at par growth of the pathogen (Table 3). The growth suppression of fungus was significantly more over the concentrations, irrespective of seed sources.

Interactions between source and concentration (SxC) revealed that Nainatikker had significantly high growth inhibition of *P. dalbergiae* at the lowest concentration of 0.5% (61.9%; Table 3). However, it had at par growth with Dehradun at the concentration of 1.0 (67.4 & 66.2%, respectively) and 2% (69.8 & 70.1% respectively). Also, Nainatikker remained at par with both Dehradun and Gyarahdevi sources at the concentration of 1.5% (68.1, 67.5, & 68.9%, respectively).

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Table 2: Effect of different concentrations of chloroform extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%) / Inhibition (%)					Mean
	0.0	**0.5**	**1.0**	**1.5**	**2.0**	
			A. alternata			
Dehradun	0.0(0.0)	44.7(50.0*)	46.3(52.3)	48.5(56.2)	49.3(57.6)	37.8(43.1)
Gyarahdevi	0.0(0.0)	41.3(43.6)	41.8(44.6)	42.8(46.2)	43.6(47.6)	34(36.4)
Nainatikker	0.0(0.0)	37.0(36.2)	38.1(38.1)	39.4(40.5)	40.6(42.4)	31(31.4)
Mean	0.0(0.0)	41.0(43.1)	42.1(45.0)	43.6(47.6)	44.5(49.1)	
SEM	**Source (S)**		**Concentration (C)**			**Interaction (SxC)**
	0.1		0.2			0.3
CD (5%)	0.4		0.5			0.8
			C. gloeosporioides			
Dehradun	0.0(0.0)	35.3(33.3)	46.1(51.9)	57.1(70.5)	61.1(76.6)	40(46.5)
Gyarahdevi	0.0(0.0)	31.1(26.6)	37.0(36.2)	44.5(49.0)	52.7(63.3)	33(35.0)
Nainatikker	0.0(0.0)	37.0(36.2)	40.0(40.5)	42.6(45.7)	47.7(54.8)	33.3(35.4)
Mean	0.0(0.0)	34.4(32.0)	40.8(42.8)	48.0(55.0)	53.9(64.9)	
SEM	**Source (S)**		**Concentration (C)**			**Interaction (SxC)**
	0.2		0.3			0.5
CD (5%)	0.6		0.8			1.4
			***Phoma* sp.**			
Dehradun	0.0(0.0)	60.8(76.2)	63.0(79.1)	64.8(81.9)	65.9(83.4)	50.9(64.1)
Gyarahdevi	0.0(0.0)	58.3(72.3)	63.1(79.5)	65.2(82.4)	69.8(88.1)	51.3(64.5)
Nainatikker	0.0(0.0)	90.0(100)	90.0(100)	90.0(100)	90.0(100)	72(80.0)
Mean	0.0(0.0)	70.0(82.8)	72.0(86.2)	73.3(88.1)	75.3(90.5)	
SEM	**Source (S)**		**Concentration (C)**			**Interaction (SxC)**
	0.1		0.2			0.3
CD (5%)	0.3		0.4			0.8

***Values in parentheses are original**

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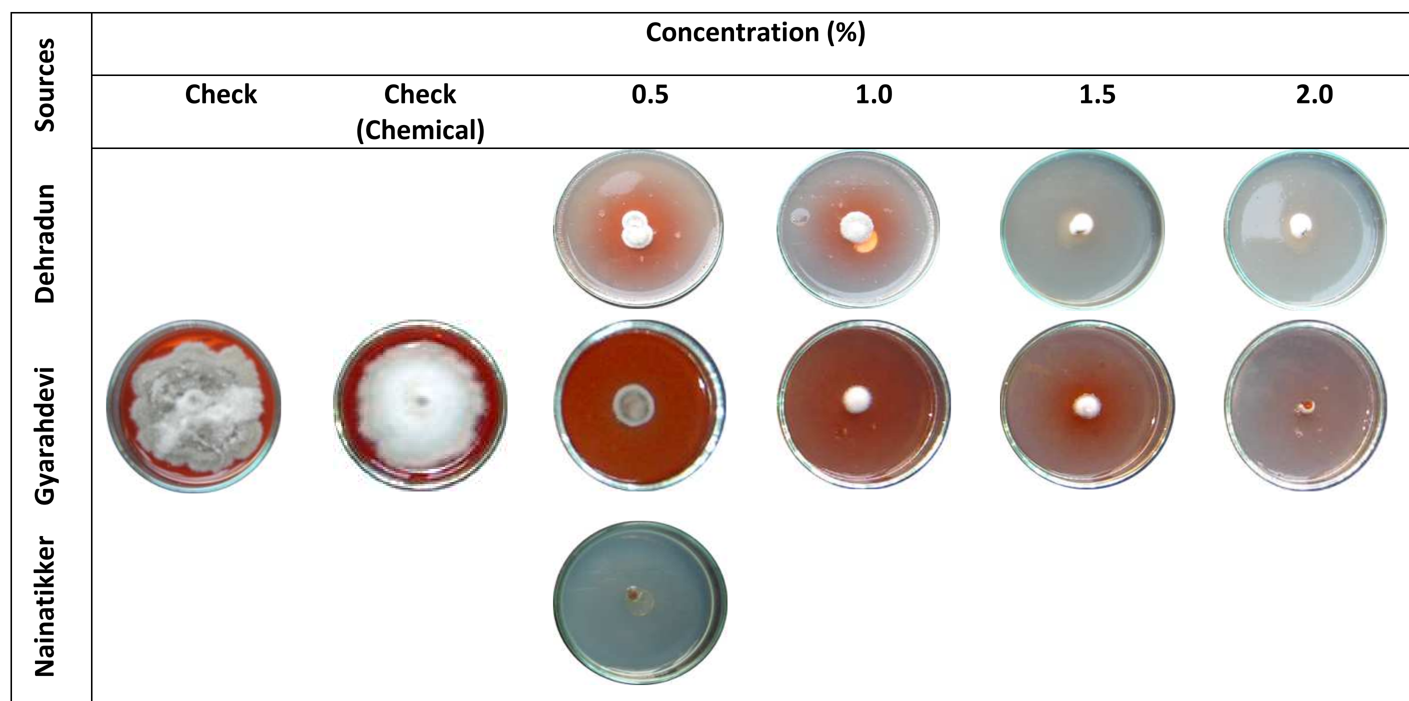

Fig. 1: Effect of chloroform extract of *S. mukorossi* from different sources on the growth of *Phoma* sp.

Irrespective of concentrations, maximum and significantly more antifungal activity was found in Dehradun source (33.0%) followed by Gyarahdevi (31.6%) and Nainatikker (29.2%) against *F. oxysporum* (Table 3). The fungus had significantly low inhibition percentage with an increase in the concentration of the chloroform extract i.e. minimum at 0.5% (34.2%) concentration and maximum at 2% (42.8%), irrespective of seed sources.

Following interactions between source and concentration (SxC), Dehradun maintained its superiority excluding 1% where it remained at par with Gyarahdevi (39.8 & 40%) and 2% with both Gyarahdevi and Nainatikker (43.4, 42.8 & 42.3%, respectively; Table 3). Also, the growth inhibition of *F. oxysporum* in Dehradun was at par with Gyarahdevi at 1% and with Gyarahdevi and Nainatikker sources at 2%.

Irrespective of concentrations, Dehradun (52.1%) had the highest reduction of growth of *G. lucidum* where as it was at par for Gyarahdevi (50.2%) and Nainatikker (50.0%) sources (Table 3). Irrespective of seed sources, growth suppression of *G. lucidum* increased significantly with rising concentrations.

Interactions between source and concentration (SxC), exhibited that Dehradun had 100% inhibition of *G. lucidum* at the concentration of 1.5% and above (Table 3). It was also observed that Gyarahdevi had significantly better growth reduction of the fungus at all the concentrations. Also, Dehradun suppressed at par growth at the lowest concentration of 0.5 (60%) and 1% (60.8%). The fungal growth inhibition at all the sources significantly increased over concentrations.

Table 3: Effect of different concentrations of chloroform extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%) / Inhibition (%)					Mean
	0.0	0.5	1.0	1.5	2.0	
			P. dalbergiae			
Dehradun	0.0(0.0)	60.0(74.3*)	66.2 (83.7)	67.5(85.3)	70.1(88.3)	52.7(66.4)
Gyarahdevi	0.0(0.0)	60.0(74.8)	62.1(78.1)	68.9(86.7)	71.3(89.7)	52.4(65.9)

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Nainatikker	0.0(0.0)	66.3(83.8)	67.4(85.2)	68.1(86.2)	69.8(88.1)	54.4(68.7)
Mean	0.0(0.0)	61.9(77.7)	65.2(82.7)	68.1(86.1)	70.4(88.7)	
	Source (S)			**Concentration (C)**		**Interaction (SxC)**
SEM	0.2			0.3		0.5
CD (5%)	0.6			0.8		1.4
				F. oxysporum		
Dehradun	0.0(0.0)	38.4(38.6)	39.8(41.0)	43.1(46.7)	43.4(47.1)	33(34.7)
Gyarahdevi	0.0(0.0)	34.4(31.9)	40.0(40.5)	41.5(43.8)	42.8(46.2)	31.6(32.5)
Nainatikker	0.0(0.0)	30.0(24.8)	34.4(31.9)	40.0(40.5)	42.3(45.2)	29.2(28.5)
Mean	0.0(0.0)	34.2(31.8)	38.0(37.8)	41.3(43.7)	42.8(46.2)	
	Source (S)			**Concentration (C)**		**Interaction (SxC)**
SEM	0.2			0.3		0.5
CD (5%)	0.6			0.8		1.3
				G. lucidum		
Dehradun	0.0(0.0)	36.0(33.8)	44.7(49.5)	90.0(100)	90.0(100)	52.1(56.7)
Gyarahdevi	0.0(0.0)	60.0(74.8)	60.8(76.2)	64.1(80.9)	66.1(83.6)	50.2(63.1)
Nainatikker	0.0(0.0)	45.3(50.5)	49.4(58.0)	53.0(63.8)	57.1(70.5)	50(48.6)
Mean	0.0(0.0)	47.0(53.0)	51.6(61.2)	69.0(81.6)	71.1(84.7)	
	Source (S)			**Concentration (C)**		**Interaction (SxC)**
SEM	0.1			0.2		0.3
CD (5%)	0.4			0.5		0.9

***Values in parentheses are original**

Irrespective of concentrations, Nainatikker (45.7%) had significant and more growth suppression against *R. solani* and lowest in Dehradun 41.3% shown in Table 3 & Fig. 2. Irrespective of seed sources, *R. solani* showed a linear and significant relationship between concentrations of extract and growth inhibition.

Interactions between source and concentration (SxC,) revealed that Nainatikker had suppressed the growth of test fungus to the maximum extent that was significantly more than other sources at all concentrations (Table 4 & Fig. 2). Moreover, Dehradun and Gyarahdevi suppressed at par growth of *R. solani* at the lowest concentration of 0.5 (48.8 & 48.5% respectively) and at 1.0 (51.3 & 50.6%, respectively), whereas, Gyarahdevi was significantly better than Dehradun at the higher concentration of 1.5 (53.9 vs 52.2%) and 2% (57.1 vs 54.4%). It was also recorded that all the sources suppressed the growth of *R. solani* significantly over changing concentrations.

It was observed in Table 4 and Fig. 3 that Nainatikker (47.1%) registered significantly more and Dehradun (37.2%) had lowest antifungal activity against *T. piluliferum* irrespective of concentrations. The growth reduction of *T. piluliferum* increased significantly with rising concentrations, irrespective of seed sources.

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Pursuing the interactions between source and concentration (SxC), it was observed that Nainatikker had significant and maximum inhibition of growth at the higher concentrations of 1.5 (60.2%) and 2% (64.1%) than other two sources (Table 4 & Fig. 3) However, Gyarahdevi performed significantly better than Dehradun at all the concentrations tested. It was also recorded that in all three sources fungal growth suppression increased significantly at the higher concentrations of 1.5% and 2%.

Table 4: Effect of different concentrations of chloroform extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%)/Inhibition (%)					Mean
	0.0	0.5	1.0	1.5	2.0	
			R. solani			
Dehradun	0.0(0.0)	48.8(56.7*)	51.3(60.9)	52.2(62.4)	54.4(66.6)	41.3(49.3)
Gyarahdevi	0.0(0.0)	48.5(56.2)	50.6(59.7)	53.9(65.2)	57.1(70.5)	42(50.3)
Nainatikker	0.0(0.0)	54.7(66.6)	56.5(69.5)	57.4(70.9)	60.0(74.8)	45.7(56.4)
Mean	0.0(0.0)	50.7(59.8)	52.8(63.4)	54.5(66.2)	57.1(70.6)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.1		0.2			0.3
CD (5%)	0.4		0.5			0.8
			T. piluliferum			
Dehradun	0.0(0.0)	42.5(45.7)	43.1(46.6)	45.8(51.4)	54.7(66.6)	37.2(42.1)
Gyarahdevi	0.0(0.0)	53.9(65.2)	56.8(69.7)	57.4(71.3)	60.8(76.2)	45.8(56.5)
Nainatikker	0.0(0.0)	54.7(66.7)	56.5(69.5)	60.2(75.3)	64.1(80.9)	47.1(58.5)
Mean	0.0(0.0)	50.4(59.2)	52.1(62.0)	54.5(65.9)	60.0(74.6)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.3			0.5
CD (5%)	0.6		0.7			1.3

***Values in parentheses are original**

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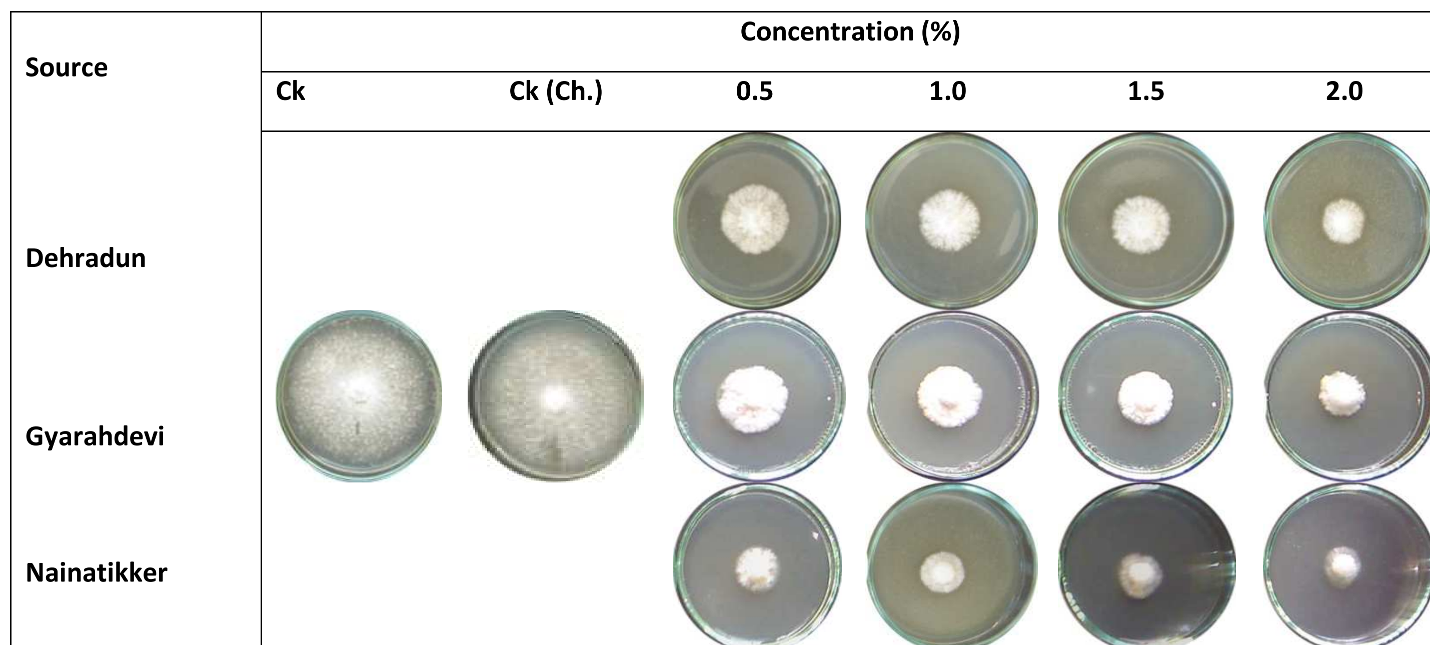
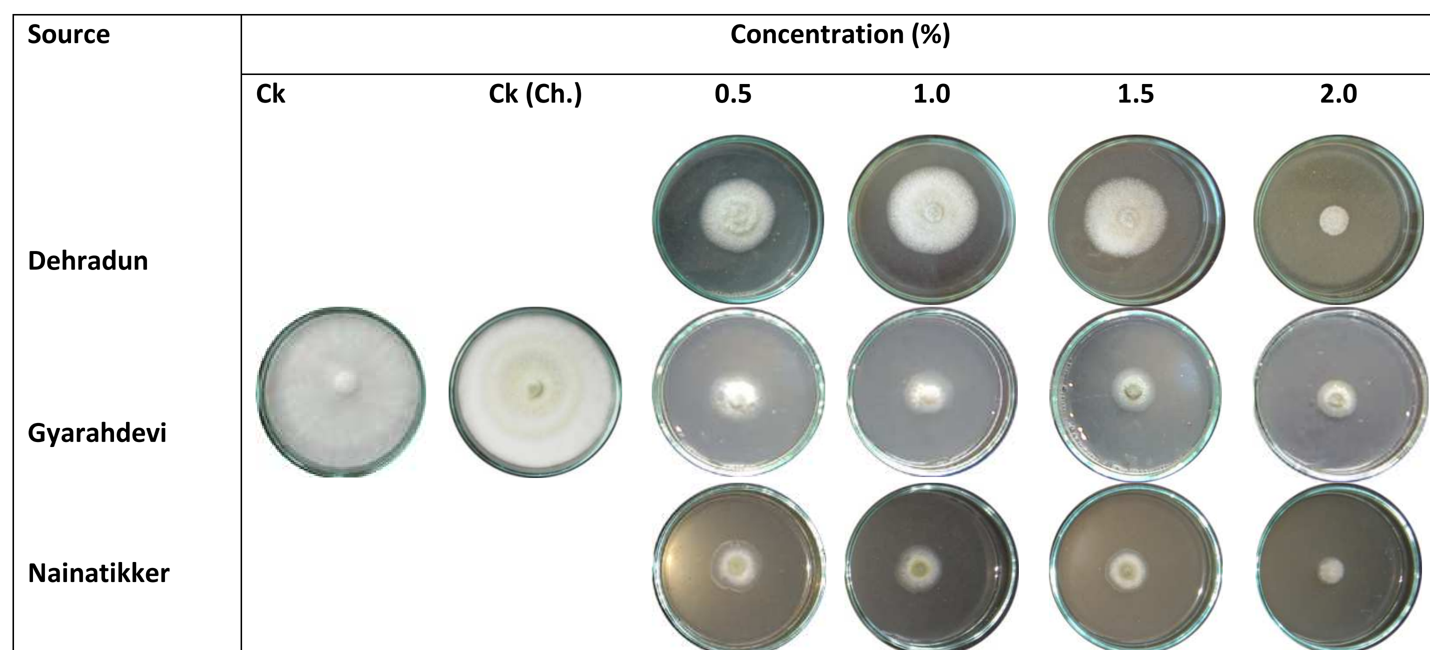


Ck= Check (Fungal culture media), Ch.= Check chemical control (Media containing chloroform)

Fig. 2: Effect of chloroform extract of *S. mukorossi* from different sources on the growth of *R. solani*



Ck= Check (Fungal culture media), Ch.= Check chemical control (Media containing chloroform)

Fig. 3: Effect of chloroform extract of *S. mukorossi* from different sources on the growth of *T. piluliferum*

Methanol extract- Table 5 revealed that irrespective of concentrations, maximum and significantly more antifungal activity was found in pericarp of seeds collected from Dehradun (33.8%) against *A. alternata* followed by Gyarahdevi (31.1%) and Nainatikker (30.1%). Irrespective of seed sources, all the concentrations of methanol extract showed significant and increasing suppression of growth i.e. maximum at concentration of 2% (42.4%) and minimum at 0.5% (36.8%).

Interactions between source and concentration (SxC) reveals that Dehradun was significantly better in suppressing the growth of *A. alternata* than other two seed sources at all concentrations except 2% of methanol extract where it remained at par with Gyarahdevi (Table 5). While Gyarahdevi had significantly more growth reduction in respect to Nainatikker at a higher concentration of 1.5% (40.4 & 38.4%) and 2% (43.7 & 40.1%). Only Gyarahdevi had significant

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suppression of *Alternaria* growth over concentrations while in rest of the two sources there were overlapping trends.

Irrespective of concentrations, Nainatikker (34.6%) and Dehradun (34.1%) had at par reduction of growth of *C. gloeosporioides* (Table 5). Irrespective of seed sources, growth suppression of *C. gloeosporioides* showed linearity over concentrations.

Interactions between source and concentration (SxC) showed that Dehradun and Nainatikker suppressed at par growth at all the concentrations of methanol extract (Table 5). Also, both of these sources remained at par with Gyarahdevi at the higher concentration of 1.5%.

The growth inhibition of *Phoma* sp. was significantly more in Gyarahdevi (68.1%) and lowest in Dehradun (52.7%) irrespective of concentrations (Table 5 & Fig. 4). It was also found, that growth inhibition from 1 to 2% was at par all the concentrations against *Phoma* sp., irrespective of sources.

Interactions between source and concentration (SxC) revealed that mycelial growth of *Phoma* sp. completely reduced to 100% from 1.0 concentration in Gyarahdevi and Nainatikker sources (Table 5 & Fig. 4). While Dehradun had minimum inhibition of the fungus with overlapping trends over concentrations.

Table 5: Effect of different concentrations of methanol extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%) / Inhibition (%)					Mean
	0.0	**0.5**	**1.0**	**1.5**	**2.0**	
			A. alternata			
Dehradun	0.0(0.0)	40.3(41.9*)	42.0(44.6)	42.8(46.2)	43.6(47.6)	33.8(36.1)
Gyarahdevi	0.0(0.0)	34.4(31.9)	37.3(36.6)	40.4(41.9)	43.7(47.1)	31.1(31.5)
Nainatikker	0.0(0.0)	35.8(34.2)	36.4(35.2)	38.4(38.5)	40.1(41.4)	30.1(29.9)
Mean	0.0(0.0)	36.8(36.0)	38.5(39.5)	40.5(42.2)	42.4(45.4)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.3		0.3			0.6
CD (5%)	0.8		1.0			1.7
			C. gloeosporioides			
Dehradun	0.0(0.0)	40.3(41.9)	42.6(45.7)	43.1(46.7)	44.7(49.5)	34.1(36.8)
Gyarahdevi	0.0(0.0)	35.5(33.8)	38.7(39.0)	43.6(47.6)	46.9(53.3)	32.9(34.8)
Nainatikker	0.0(0.0)	40.3(41.9)	42.5(45.7)	44.2(48.5)	45.8(51.4)	34.6(37.5)
Mean	0.0(0.0)	38.7(39.2)	41.3(43.5)	43.6(47.6)	45.8(51.4)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.3		0.3			0.6
CD (5%)	0.7		1.0			1.7
			***Phoma* sp.**			
Dehradun	0.0(0.0)	64.8(81.9)	65.2(82.4)	66.3(83.8)	67.0(84.8)	52.7(66.6)
Gyarahdevi	0.0(0.0)	70.7(89.1)	90.0(100)	90.0(100)	90.0(100)	68.1(77.8)
Nainatikker	0.0(0.0)	66.3(83.8)	90.0(100)	90.0(100)	90.0(100)	67.3(76.8)
Mean	0.0(0.0)	67.3(84.9)	81.7(94.1)	82.1(94.6)	82.3(94.9)	

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	Source (S)	Concentration (C)	Interaction (SxC)
SEM	0.2	0.3	0.4
CD (5%)	0.6	0.7	1.3

***Values in parentheses are original**

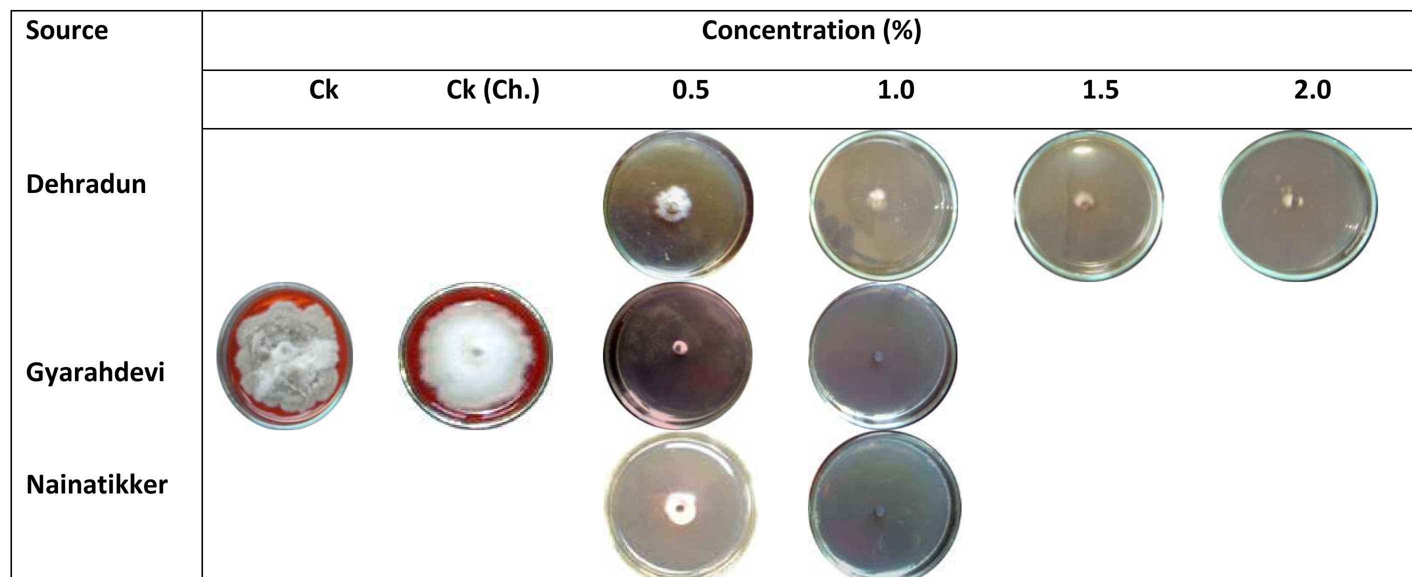

Fig. 4: Effect of methanol extract of *S. mukorossi* from different sources on the growth of *Phoma* sp.

Irrespective of concentrations, Gyarahdevi had maximum and significantly higher growth inhibition of 68.2% while Dehradun had lowest of 55.6% against *P. dalbergiae* (Table 6). Irrespective of sources, suppression of mycelial growth of *P. dalbergiae* was at par at 1% and 1.5 % concentration.

Following interactions between source and concentration (SxC), it was concluded that Gyarahdevi had 100% antifungal activity at the concentration of 1.0 against *P. dalbergiae* (Table 6). Barring the concentration of 1.0, Nainatikker was at par with Dehradun at 0.5, 1.5 and 2.0 concentrations. Also, Dehradun and Nainatikker were at par at the lower concentrations of 0.5 and 1.0 and at the higher of 1.5 and 2.0.

It was observed in Table 6 that Gyarahdevi (33.7%) registered significantly more and Dehradun (27.7%) had lowest antifungal activity against *F. oxysporum*, irrespective of concentrations. The growth reduction of the fungus increased significantly with rising concentrations, irrespective of seed sources.

Interactions between source and concentration (SxC) revealed that Gyarahdevi had significantly better growth suppression at all concentrations (Table 6). Also, it remained at par with Nainatikker at the highest concentration of 2% (43.6 & 42.6%).

Moreover, Nainatikker was second to highest and was at par at the concentration of 1.0 and 1.5.

Table 6 showed significantly differential growth suppression of *G. lucidum* by pericarp of seeds collected from different sources, for example, Nainatikker had highest (45.0%) and Dehradun had lowest of 37.3%, irrespective of concentrations. *G. lucidum* showed linear and significant relationship between concentrations of extract and growth inhibition, irrespective of sources. Interactions between source and concentration (SxC) revealed that Nainatikker had highest growth suppression of *G. lucidum* at all the concentrations barring 2% concentration of Dehradun source where 61.7% of growth inhibition was observed (Table 6). At lower concentrations up to 1.5 Gyarahdevi performed better than Dehradun while at higher concentration of 1.5% and 2% Dehradun scored significantly better than Gyarahdevi. The antifungal activity of Gyarahdevi had overlapping trends over concentrations, while the rest of the two recorded significant suppression with increasing concentrations.

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Table 6: Effect of different concentrations of methanol extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%) / Inhibition (%)					Mean
	0.0	0.5	1.0	1.5	2.0	
			P. dalbergiae			
Dehradun	0.0(0.0)	68.2(86.2*)	69.0(87.1)	69.8(88.1)	70.7(89.0)	55.6(70.1)
Gyarahdevi	0.0(0.0)	71.1(89.5)	90.0(100)	90.0(100)	90.0(100)	68.2(77.9)
Nainatikker	0.0(0.0)	69.4(87.6)	69.8(88.1)	70.7(89.0)	71.6(90.0)	56.3(71.0)
Mean	0.0(0.0)	70.0(87.8)	76.3(91.7)	76.8(92.4)	77.4(93.0)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.3			0.4
CD (5%)	0.6		0.7			1.2
			F. oxysporum			
Dehradun	0.0(0.0)	31.7(27.6)	33.5(30.5)	34.7(32.4)	38.7(39.0)	27.7(25.9)
Gyarahdevi	0.0(0.0)	40.3(41.9)	41.5(43.8)	42.8(46.2)	43.6(47.6)	33.7(35.9)
Nainatikker	0.0(0.0)	37.0(35.7)	40.1(41.4)	40.6(42.4)	42.6(45.7)	32(33.1)
Mean	0.0(0.0)	36.3(35.1)	38.3(38.6)	39.4(40.3)	41.6(44.1)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.2			0.4
CD (5%)	0.5		0.6			1.0
			G. lucidum			
Dehradun	0.0(0.0)	32.6(29.0)	35.6(33.8)	56.4(69.3)	61.7(77.6)	37.3(42.0)
Gyarahdevi	0.0(0.0)	50.8(60.1)	51.3(60.9)	52.7(63.3)	53.6(64.7	41.7(49.8)
Nainatikker	0.0(0.0)	52.4(62.9)	55.1(67.3)	57.7(71.4)	60.0(74.3)	45(55.2)
Mean	0.0(0.0)	45.3(50.7)	47.3(54.0)	55.6(68.0)	58.3(72.2)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.2			0.4
CD (5%)	0.5		0.6			1.1

***Values in parentheses are original**

Irrespective of concentrations, Nainatikker had maximum and significantly more growth reduction of 48% followed by Dehradun (44.8%) and Gyarahdevi (41.6%, Table 7 & Fig. 5) sources against *R. solani*. All the concentrations had linear and significant reduction of growth of *R. solani*, irrespective of sources.

Interactions between source and concentration (SxC) revealed that Nainatikker had significantly better growth suppression at all concentrations followed by Dehradun and Gyarahdevi sources (Table 7 & Fig. 5). It was also recorded that Gyarahdevi and Nainatikker had overlapping trends among concentrations.

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Irrespective of concentrations, Gyarahdevi had significant and maximum inhibition of 50% of *T. piluliferum* followed by Nainatikker (46.0%) and Dehradun (44.9%; Table 7 & Fig.6) sources. Irrespective of sources, *T. piluliferum* had significant and linear growth reduction over concentrations.

Interactions between source and concentration (SxC), showed that the performance of Gyarahdevi was significantly better than other two sources and at all the concentrations and Nainatikker and Dehradun had at par growth of *T. piluliferum* at all concentrations barring 2% where Nainatikker performed significantly better than Dehradun (Table 7 & Fig. 6) Barring the concentration of 2.0, all the sources had overlapping trends among concentrations.

Table 7: Effect of different concentrations of methanol extract of *S. mukorossi* on radial growth of test fungi

Source	Concentration (%) / Inhibition (%)					Mean
	0.0	**0.5**	**1.0**	**1.5**	**2.0**	
			R. solani			
Dehradun	0.0(0.0)	54.0(64.8)*	55.4(67.6)	57.1(70.5)	58.0(71.9)	44.8(54.9)
Gyarahdevi	0.0(0.0)	50.8(60.0)	51.6(61.4)	52.4(62.8)	53.4(64.3)	41.6(49.7)
Nainatikker	0.0(0.0)	57.7(71.4)	60.0(74.3)	60.2(75.2)	62.0(78.1)	48(59.8)
Mean	0.0(0.0)	54.0(65.4)	56.0(67.7)	57.0(69.5)	57.8(71.4)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.3			0.6
CD (5%)	0.7		0.9			1.6
			T. piluliferum			
Dehradun	0.0(0.0)	54.0(64.7)	55.3(67.6)	57.1(70.5)	58.3(72.3)	44.9(55.0)
Gyarahdevi	0.0(0.0)	60.5(75.7)	61.4(77.1)	62.4(78.6)	63.4(80.0)	50.0(62.3)
Nainatikker	0.0(0.0)	55.1(67.3)	56.0(68.7)	57.0(70.1)	60.0(74.3)	46.0(56.1)
Mean	0.0(0.0)	56.4(69.2)	57.6(71.1)	58.8(73.0)	60.4(75.6)	
	Source (S)		**Concentration (C)**			**Interaction (SxC)**
SEM	0.2		0.3			0.5
CD (5%)	0.7		0.9			1.5

***Values in parentheses are original**

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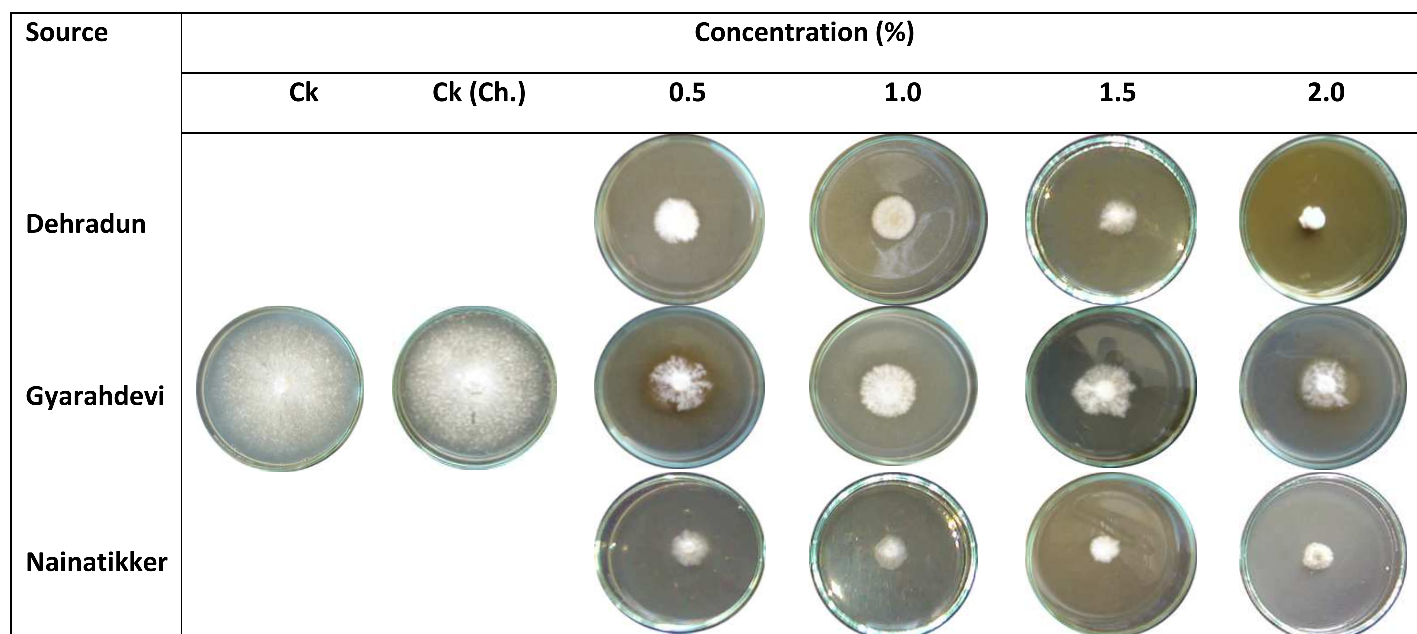

Fig. 5: Effect of methanol extract of *S. mukorossi* from different sources on the growth of *R. solani*

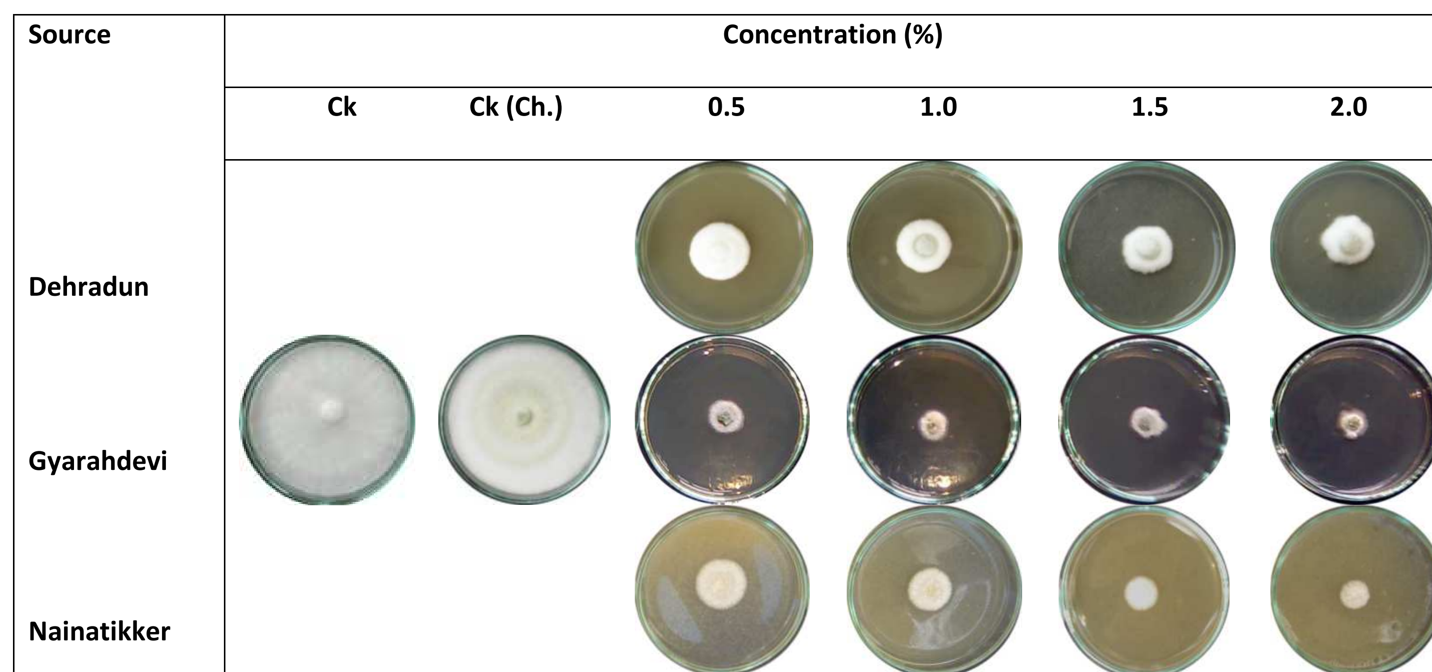

Fig. 6: Effect of methanol extract of *S. mukorossi* from different sources on the growth of *T. piluliferum*

DISCUSSION

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer [13,14]. Regarding chloroform extract, the current study found that this extract of all the three sources presented IC_{50} at all concentrations against only two fungi namely, *Phoma* sp. and *P. dalbergiae.* Similar to the present study, ethanol and chloroform extracts of *S. mukorossi* has shown antibacterial activity against *H. pylori* [8] at very low concentrations (10 µg/ml for both extracts). In a subsequent study, Tsuzuki verified that the crude extracts (hydroalcoholic and butanol) of *S. saponaria* showed strong antifungal activity against clinical isolates of yeasts *C. albicans* [15]. Focusing on methanol extract, we have observed that this extract of all the three sources has attained IC_{50} at all concentrations against four fungi namely, *Phoma* sp., *P. dalbergiae, R. solani* and *T. piluliferum.* Previous studies had reported that ethyl acetate extract from endophytes of *S. Saponaria* showed a greater antimicrobial activity against some

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pathogenic bacteria but methanol extract did not show positive results for human pathogenic bacteria [16]. Results concluded that the methanol extract of different sources performed much better than chloroform extract barring one fungus, *G. lucidum* (1.5%); all other fungi exhibited IC_{50} at their minimum concentration of 0.5%. Predominantly, antifungal activity of pericarp extracts (chloroform and methanol) of *S. mukorossi* was never tested against forest fungi though it was a popular tree under intensive uses.

CONCLUSIONS

The results of preliminary screening suggested that chloroform extract of all three sources could not achieve IC_{50} against *A. alternata* and *F. oxysporum* ranging from 0.5% to 2% concentration. Further, it was common between both extracts of all three sources registered IC_{50} at all concentrations against Phoma sp. and *P. dalbergiae*.

Future studies concerning antimicrobial activities must be carried out with other extracts (petroleum ether, butanol etc.) of fruit pericarp of *S. mukorossi* against forest fungi to justify their antifungal properties.

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Data collection- Dr. Prerana Badoni
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Literature search- Dr. Prerana Badoni
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Critical review- Dr. Y. P. Singh
Article editing- Dr. Prerana Badoni, Dr. Kartik Uniyal
Final approval- Dr. Y. P. Singh

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