The Journal of Phytopharmacology 2018; 7(3): 230-241
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(3): 230-241
May- June
Received: 15-02-2018
Accepted: 26-04-2018

Savita Joshi
Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263001, Uttarakhand, India

Parikshit Kumar
Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263001, Uttarakhand, India

Prabha Pant
Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263001, Uttarakhand, India

SC Sati
Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263001, Uttarakhand, India

Correspondence:
Parikshit Kumar
Department of Botany, D.S.B. Campus, Kumaun University, Nainital-263001, Uttarakhand, India
Email: pk2461989[at]gmail.com

A comparative evaluation of Kumaun Himalayan Gymnosperms for their Antifungal potential against plant pathogenic fungi

Savita Joshi, Parikshit Kumar*, Prabha Pant, SC Sati

ABSTRACT

Fungicidal activity of 10 ethnobotanically known Kumaun Himalayan gymnospermous plants namely *Araucaria cunninghamii*, *Biota orientalis*, *Cedrus deodara*, *Cephalotaxus griffithi*, *Cryptomeria japonica Cupressus torulosa*, *Ginkgo biloba*, *Juniperus communis*, *Picea smithiana* and *Pinus wallichiana* were tested against six plant disease causing fungal pathogens by agar well-diffusion method. Forty extracts of these gymnospermic leaves in different organic solvents (methanol, ethanol, chloroform and hexane) were studied by performing the 160 sets of experiments. The MIC values of each extract (where % inhibition ≥ 40%) were also determined. All the plant extracts exhibited strong antifungal activity. Results indicated that all leaves extracts of *C. griffithi* and *G. biloba* were found most effective among the tested plants extracts. Hexane extract of *C. griffithi* was showed highest inhibitory activity against *C. falcatum* (72%; MIC, 7.81µg/ml) and *T. indica* (70%; MIC, 15.62µg/ml). On the other hand, ethanol extract of *G. biloba* also showed remarkable activity against *P. oryzae* (66% with MIC, 7.81g/ml). While *P. wallichiana* leave extracts were found less active among the studied plants against all the tested fungal strains. The chloroform extracts were found the most effective against all the tested fungi (10% to 60%), followed by ethanol extract (30-50%), methanol extract (20-40%), while in hexane extracts ranged 10-30% only. The extracts of *C. griffithi* exhibited superior Relative Antifungal Activity (RAA, 20%), followed by *G. biloba* and *A. cunninghamii* (RAA, 19 and 12%, respectively). All data were also analyzed for determination of total activity of plant for each studied species of gymnosperm. *C. griffithi* had maximum activity i.e. 71 % followed by *G. biloba* (54%) and *A. cunninghamii* (33%). *C. torulosa* showed the least total activity and RAA i.e. 8% and 3%, respectively. All the plant species assayed possess definite antifungal properties and suggested for phytochemical analysis to identify the active principles responsible for their antifungal activity.

Keywords: Antifungal activity, Gymnosperms, Plant extracts, MIC, RAA.

INTRODUCTION

Plants extracts and their various other forms, are being used for centuries in different traditional systems of medicine for the treatment of human ailments [1]. Their use against plant pathogens, though a relatively recent practice has gained momentum due to the well-known problem associated with the use of synthetic pesticides. The use of synthetic chemicals as antimicrobial for the management of plant diseases has undoubtly increased crop protection but with considerable deterioration of environmental quality and human health [2]. A large number of plants have been reported to possess fungi toxic properties against plant pathogens which could be exploited commercially with practically no residual or toxic effect on ecosystem [3]. The effective plant constituents can combat human and plant pathogenic bacteria, fungi and viruses without toxic side effects and environmental hazards. This is the reason that's why search for plant products having antimicrobial properties is being intensified in recent years. Plants have supplied over 25% of prescription drugs used in human medicine and such pharmacologically active plants have also provided leads to natural pesticides [4]. Today, several fungi that colonize crop plants may often severally affect human health by production of mycotoxins, e.g., the trichothecenes from *Fusarium* on wheat. Mycotoxins constitute an additional reason why fungal control on crop plants is mandatory [5].

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life [6]. In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful

effects of pesticides on human health and the environment [7]. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. It is imperative to develop antifungal strategies based on new classes of molecules, which act through new sites and/or mechanisms [8-10]. The discovery of new antifungal agents remains an important challenge for the scientific community and traditional medicinal plants may furnish promising material for the development of antifungal drugs [11, 12].

The utilization of plant extracts which are natural sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or environmental problems at any concentration which they are used [13, 14]. In agriculture, the crop loss due to plant pathogens has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases [15]. Crude extracts of some well-known medicinal plants are used to control some of the plants pathogens [16]. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world [17, 18] but antifungal therapy is playing a greater role in health care and the screening of traditional plants in search of novel antifungal is now more frequently performed [19]. Further work is required to isolate the bioactive constituents and test the antifungal properties of these compounds, this may help to find the compound(s) responsible for antifungal activity.

In folk medicines some gymnospermic plants are being used as an antimalarial, antirheumatic, abortifacient and antibronchitis [20-22] as well as antiasthmatic [23]. Erdemoglu and Sener suggested that gymnosperms possess various biological activities such as antimicrobial, anti-inflammatory, anticancer and antioxidant [24].

Undoubtedly, gymnosperms are the reservoir of chemotherapeutants providing an unlimited source of new medicinal compounds [25, 26]. They are used traditionally throughout the world to treat many ailments [27, 28]. The recent finding reported that gymnospermous plants contain various secondary metabolites such as tannins, terpenoids, flavonoids, alkaloids, glycosides, ligans, phenol, steroid and sugar derivatives [28, 29]. These phytochemicals have antifungal properties and they serve as defence agent against plant pathogenic microorganisms [30, 31]. Himalaya's extraordinarily rich flora and its information on medicinal plants are well documented. In the present study the potential of Himalayan medicinal plants as a resource for new biofungicides is being investigated. A detail comparative evaluation of 10 Kumaun Himalayan gymnosperms for their antifungal potential against six plant pathogenic fungi is made.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of selected plants were collected between March and June from various areas of Nainital district, Kumaun Himalaya, India. Plant parts were collected on the basis of their ethnobotanical survey. The plant specimens were identified in the Botany Department, D.S.B. Campus, Kumaun University, Nainital. Voucher specimens were deposited in the herbarium of the department, for further reference. Each specimen/plant material was labelled, numbered, a noted with the date of collection, locality, and their medicinal uses were recorded.

Plant extraction

Leaves of the identified plants were thoroughly washed with distilled water and dried at the room temperature (20±2°C). The dried material was powdered in an electric grinder. To prepare stock solution 50g of this powder was placed in a 500 ml conical flask mixed with 200ml of solvents (w/v, 50g/200ml). The mouth of flasks are tightly plugged with non-absorbent cotton and tightly wrapped with aluminium foil to prevent evaporation. Solvents used for extraction were methanol, ethanol, chloroform, hexane and distilled water. All mixtures were shaken on a rotary incubator shaker at 190-220 rpm for 48 h at 37° C. The mixtures were filtered through Whatman filter paper no.1 and the filtrate collected separately in a clean beaker. The extracts were evaporated, using steam bath to dryness at 40° C. The dry extracts were weighed and kept in sterile sample bottles and stored in the refrigerator at 4° C for further use.

Microorganisms used

Six fungal strains (*Alternaria alternata, Colletotrichum falcatum, Fusarium oxysporum, Pyricularia oryzae*, *Sclerotinia rolfsii* and *Tillatia indica*) were obtained from Plant Pathology Department, Pantnagar University, Pantnagar, which were previously isolated from diseased plant materials. The fungal cultures were prepared in potato dextrose agar (PDA) (Hi Media Laboratories Ltd., Bombay) plates and incubated at 25ºC for 48-72 h. After incubation, the culture tubes were stored at 4ºC for further experiment.

Antifungal Screening

The antifungal activity of different extracts was tested against seven fungal strains employing the 'Agar well' technique of Grover and Moore [32]. Potato Dextrose Agar (Hi Media, 39 gm of medium dissolved in 1000 ml of distilled water) was used. The medium was autoclaved at 120° C for 30 minutes. 20ml of PDA media was poured into the 90mm petri plates. After solidification of agar plates, the appropriate well was made on agar plate by using cork borer of size 7.0mm and 200 µl of the extract was added into each well. Mycelial disks (7mm diam.) of actively growing colonies of tested fungal strains were cut from the periphery of the culture plates and aseptically placed 2.5 cm apart from the wells in the assay plates (90mm diam.). A standard antibiotic clotrimazole was used as positive control in the experiment. The tests were performed in triplicates and these plates were incubated at 25 ± 2°C for 4 to 5 days.

Minimum Inhibitory Concentration (MIC) evaluation:

The MIC was evaluated on plant extracts that showed 40% inhibition at 1,000 µg/ml. This test was performed at seven concentration of extract (500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/ml) employing the same 'agar well' technique.

Observation and Reading of Antifungal Activity:

The antifungal activity of the plant extracts was analyzed by measuring the radial growth of the test fungi after 4-5 days of incubation in 2 directions: R_1 (radius in opposite direction of well)

and R_2 (radius in direction of the well filled with plant extract). Percent inhibition of radial growth was calculated as suggested by Sati and Joshi [33] and expressed as mean value with standard error of means (SEM).

$$\text{Percent Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

Statistical Analysis

Each plant extract was tested in three replicates and all the data were recorded separately for each plant extract and microbes. Altogether 240 sets of experiments were conducted for all plant extracts (10 plants species extracts in 4 solvents tested against 6 fungi). These results were statistically analyzed for Standard Error of Mean (SEM) and determination of Total Activity of plant and Relative Antifungal Activity (RAA) by using following formula:

$$\text{Total percent activity of plant} = \frac{\text{Activity observed (AO)}}{\text{Activity tested (AT)}} \times 100$$

$$\text{Relative antifungal activity (RAA)} = \frac{\text{Total activity of plant}}{\text{Total AFVI}} \times 100$$

The experimental results pertaining to the antifungal activity were expressed as the mean ± standard error of the mean (SEM). On the basis of percent inhibition antifungal activity of selected plants extracts (methanol, ethanol, chloroform and hexane extract) against six tested fungi four Hierarchical Clusters are developed by using SPSS software (version 16 for window) to find out the closeness between plants extracts activity.

RESULTS

The results of present investigation revealed that the leaves extracts of all studied plant species possess antifungal activity against all tested plant pathogenic fungal strains (Table 1). The leaves extracts of *C. griffithi* were found most effective among the tested plants extracts. The highest relative antifungal activity was found for *C. griffithi* (RAA, 25%). Hexane extract of *C. griffithi* showed highest inhibitory activity against *C. falcatum* (72% with MIC, 7.81µg/ml) and *T. indica* (70% with MIC, 15.62) followed by chloroform extract against *T. indica* (67%), ethanol extract against *C. falcatum* (64%) and *P. oryzae* and *T. indica* 62% each. While methanol extract of *C. griffithi* showed its highest activity against *S. rolfsii* (66%), followed by *C. falcatum* (65%), for *A. alternata* and *P. oryzae* the extract showed percent inhibition 64% each. It is interesting to note that almost all the extract showed their higher activity against the fungal pathogens in comparison to the standard synthetic antifungal agent clotrimazole, used as positive control (Table 1). The second highest fungitoxic activity was found for *G. biloba* (RAA, 21%). Different extracts of *G. biloba* also showed remarkable antifungal activity against tested pathogenic fungi at 1000 µg/ml. As evident from table 1 that all the extracts of *G. biloba* leaves showed a very significant activity against the pathogen used except *F. oxysporum* (Fig 1). Methanol extract of *G. biloba* showed highest activity compare to other extracts of *G. biloba*. Table 1 indicates that, this extract showed highest activity against *P. oryzae* having percent inhibition, 67 % followed by *A. alternata* showing percent inhibition 63%, *T. indica* (57 %), *C. falcatum* (56 %). However a moderate activity was observed against *S. rolfsii* (49 %). Ethanol extract of *G. biloba* also showed significant fungitoxic activity. Its highest activity was found against *P. oryzae* (66 %) followed by *C. falcatum* (65 %), *A. alternata* (60 %) and *T. indica* (55 %).

Relying upon table 1, *S. rolfsii* which was remained more resistant to methanol and ethanol extract of *G. biloba* but found very sensitive in chloroform and hexane extracts of *G. biloba*. Similarly, *T. indica* was found less sensitive in methanol, ethanol and chloroform extracts but it was the most sensitive for hexane extract of *G. biloba.* It is very outstanding observation that most of the leave extracts of *G. biloba* showed better inhibitory activity than Clotrimazol, a standard antifungal agent used as positive control in this investigation (Table 1).

All the extracts of *P. wallichiana* showed less antifungal activity as compared to other plant extracts. Out of six fungi tested, four strains were not inhibited by any of the fraction of *P. wallichiana*. Ethanol and methanol extract of *P. wallichiana* showed its highest activity against *S. rolfsii* (64 % and 58%, respectively).

The lowest MIC value was observed in two cases first in hexane extract of *C. griffithi* against *C. falcatum* and secondly in ethanol extract of *G. biloba* against *P. oryzae* (7.81µg/ml in both cases). The second lowest MIC value (15.62µg/ml) was found in13 cases for all tested plant extracts. As shown in the table 2, *C. griffithi* and *G. biloba* leaves extracts performed remarkable antifungal activity against all pathogenic fungi except *F. oxysporum*. On the other hand all extracts of *P. wallichiana* and *C. deodara* leaves were found weak active against tested fungi (Table 2).

The calculated value for total activity of plant and Relative antifungal activities of each plant is tabulated in table 3. The bioactivity of each used gymnosperms against test microbes with respect to the solvent applied is also summarized in table 3.

As evident from table 3, that most of the extracts of these 10 tested plants showed a significant antifungal activity (percent inhibition ≥50%). All the studied plants had varied activity with the maximum total activity, 71% observed for *C. griffithi*, as out of 24 tests (AT= 24, 4 extracts against 6 fungi) at 17 places (AO=17) extracts of this plant was showed antifungal potential. Whereas, the total activity for *G. biloba* was also found very effective (54%) followed by *A. cunninghamii* (33%) and *C. japonica* (29%). On the other hand, *B. orientalis* and *P. smithiana* both Kumaun Himalayan Gymnosperms demonstrated the same total activity (21% for each plant). The lowest activity was observed for *C. deodara* (total activity, 8%). The highest relative activity value observed for *C. griffithi* is 26% followed by *G. biloba* (RAA, 19%) and *A. cunninghamii* (RAA, 12%) (Fig. 3). The activity order of the studied plants are as *C. griffithi* > *G. biloba* > *A. cunninghamii* > *J. communis* > *C. japonica* > *C. torulosa* > *B. orientalis* > *P. smithiana* > *C. deodara* > *P. wallichiana* (Fig. 2&3).

Table 1: Antifungal potential of different extracts of studied gymnosperms

S. No.	Botanical name/family	Extracts	Antifungal activity of gymnospermic plants (Percent Inhibition)					
			A. alternata	*C. falcatum*	*F. oxysporum*	*P. oryzae*	*S. rolfsii*	*T. indica*
1.	*A. cunninghamii* (Araucariaceae)	Methanol	42±0.6	40±1.5	30±8.0	41±1.0	38±3.0	43±0.6
		Ethanol	37±4.6	51±3.5	27±3.8	51±1.0	52±3.0	43±0.0
		Chloroform	39±3.2	47±4.5	na	42±1.0	51±0.8	32±0.0
		Hexane	57±3.3	47±7.0	na	52±2.8	53±0.8	51±0.8
2.	*B. orientalis* (Cuppreaceae)	Methanol	42±2.0	27±2.3	39±3.4	45±1.3	57±0.8	na
		Ethanol	58±1.0	27±6.6	39±1.2	47±7.0	59±2.3	na
		Chloroform	na	na	na	Na	58±0.6	na
		Hexane	58±2.9	na	na	31±1.3	47±4.5	na
3.	*C. deodara* (Pinaceae)	Methanol	67±0.0	na	53±0.3	49±2.4	46±3.5	39±1.5
		Ethanol	48±1.2	na	45±3.3	49±1.0	na	45±2.0
		Chloroform	na	na	na	Na	na	na
		Hexane	na	na	na	Na	na	na
4.	*C. griffithi* (Cephalotaxaceae)	Methanol	64±2.3	65±9.4	na	64±0.0	66±1	62±3.0
		Ethanol	59±2.3	64±0.3	na	62±2.0	46±5.6	62±3.0
		Chloroform	58±2.0	63±1.3	na	57±2.6	45±2.8	67±2.0
		Hexane	62±2.3	72±1.3	na	64±3.6	na	70±2.3
5.	*C. japonica* (Taxodiaceae)	Methanol	42±1.0	26±1.2	27±0.3	39±0.6	45±1.6	na
		Ethanol	59±2.4	64±2.5	na	62±2.0	46±1.5	62±2.0
		Chloroform	46±1.2	na	54±2.0	Na	52±1.3	na
		Hexane	58±1.2	na	na	Na	31±1.0	na
6.	*C. torulosa* (Cupressaceae)	Methanol	49±1.0	34±4.6	33±1.3	33±2.3	49±3.0	na
		Ethanol	46±1.8	40±2.0	na	27±6.6	51±1.0	na
		Chloroform	46±3.5	47±3.3	32±0.0	Na	63±2.0	na
		Hexane	39±3.6	51±0.6	na	Na	42±1.0	na
7.	*G. biloba* (Ginkgoaceae)	Methanol	63±2.0	56±5.3	na	67±2.3	49±2.0	57±2.6
		Ethanol	60±0.0	65±2.3	na	66±1.3	42±6.7	55±5.5
		Chloroform	49±1.3	58±4.2	na	54±4.3	62±7.6	46±3.5
		Hexane	47±3.3	na	na	Na	53±2.4	67±0.0
8.	*J. communis* (Cupressaceae)	Methanol	na	54±3.0	na	Na	51±0.6	42±6.7
		Ethanol	na	50±3.8	na	53±0.3	53±2.6	42±1.8
		Chloroform	na	32±3.3	na	29±3.3	29±3.3	na
		Hexane	na	43±1.3	na	Na	29±4.3	na
9.	*P. smithiana* (Pinaceae)	Methanol	47±3.5	na	52±6.4	Na	49±4.6	na
		Ethanol	50±0.6	na	43±0.0	Na	46±3.0	na
		Chloroform	46±4.3	na	54±1.7	Na	52±2.1	na
		Hexane	52±2.0	na	44±1.3	Na	47±2.6	na
10.	*P. wallichiana* (Pinaceae)	Methanol	na	25±3.0	na	Na	58±3.9	na
		Ethanol	na	27±5.3	na	Na	64±3.7	na
		Chloroform	na	32±3.7	na	Na	na	na
		Hexane	na	50±0.0	na	Na	na	na
	Positive control (Clotrimazole)		40±1.3	40±3.0	45±1.0	48±4.5	45±1.5	48±1.0

na: not active

Table 2: Minimal inhibitory concentration (MIC) of crude extracts from plant species against plant pathogens (based on Inhibition ≥ 40%)

S. No.	Plants	Extracts	Minimal inhibitory concentration (µg/ml)					
			A. alternata	*C. falcatum*	*F. oxysporum*	*P. oryzae*	*S. rolfsii*	*T. indica*
1.	*A. cunninghamii*	Methanol	250	na	na	125	nt	125
		Ethanol	nt	125	nt	62.5	62.5	na
		Chloroform	nt	125	nt	250	62.5	nt
		Hexane	125	62.5	nt	125	31.25	62.5
2.	*B. orientalis*	Methanol	62.5	nt	nt	125	62.5	nt
		Ethanol	31.25	nt	nt	62.5	15.62	nt
		Chloroform	nt	nt	nt	Nt	15.62	nt
		Hexane	31.25	nt	nt	Na	125	nt
3.	*C. deodara*	Methanol	31.25	nt	125	125	250	nt
		Ethanol	500	nt	250	250	nt	250
		Chloroform	nt	nt	nt	Nt	nt	nt
		Hexane	nt	nt	nt	Nt	nt	nt
4.	*C. griffithi*	Methanol	31.25	31.25	nt	15.62	15.62	31.25
		Ethanol	62.5	15.62	nt	31.25	500	31.25
		Chloroform	62.5	31.25	nt	125	250	15.62
		Hexane	31.25	15.62	nt	31.25	nt	7.81
5.	*C. japonica*	Methanol	na	nt	nt	Nt	500	nt
		Ethanol	62.5	125	nt	125	250	62.5
		Chloroform	250	nt	125	Nt	125	nt
		Hexane	15.62	nt	nt	Nt	nt	nt
6.	*C. torulosa*	Methanol	125	nt	nt	Nt	500	nt
		Ethanol	na	125	nt	Nt	31.25	nt
		Chloroform	250	62.5	nt	Nt	62.5	nt
		Hexane	nt	125	nt	Nt	na	nt
7.	*G. biloba*	Methanol	31.25	125	nt	15.62	na	31.25
		Ethanol	15.62	15.62	nt	7.81	250	31.25
		Chloroform	125	62.5	nt	125	62.5	125
		Hexane	125	nt	nt	Nt	125	15.62
8.	*J. communis*	Methanol	nt	125	nt	Nt	31.25	250
		Ethanol	nt	62.5	nt	62.5	62.5	na
		Chloroform	nt	nt	nt	Nt	nt	nt
		Hexane	nt	nt	nt	Nt	nt	nt
9	*P. smithiana*	Methanol	125	nt	250	Nt	250	nt
		Ethanol	62.5	nt	62.5	Nt	na	nt
		Chloroform	250	nt	125	Nt	62.5	nt
		Hexane	125	nt	250	Nt	125	nt
10.	*P. wallichiana*	Methanol	nt	nt	nt	Nt	31.25	nt
		Ethanol	nt	nt	nt	Nt	15.62	nt
		Chloroform	nt	nt	nt	Nt	nt	nt
		Hexane	nt	125	nt	Nt	nt	nt
Positive control (Clotrimazole)			7.81	7.81	3.9	1.95	1.95	1.95

na: not active; nt: not tested

Table 3: Antifungal potential of different extracts of studied gymnosperms (based on Inhibition ≥ 50%)

Plants	*A. alternata*	*C. falcatum*	*F. oxysporum*	*P. oryzae*	*S. rolfsii*	*T. indica*	Activity observed (AO)	Total activity of plant
1. *Araucaria cunninghamii*	H	E	-	H, E	H, C, E	H	8	33
2. *Biota orientalis*	H, E	-	-	-	C, E, M	-	5	21
3. *Cedrus deodara*	M	-	M	-	-	-	2	8
4. *Cephalotaxus griffithi*	H, C, E, M	H, C, E, M	-	H, C, E, M	M	H, C, E, M	17	71
5. *Cryptomeria japonica*	H, E	E	C	E	C	E	7	29
6. *Cupressus torulosa*	-	H	-	-	C, E	-	3	12
7. *Ginkgo biloba*	E, M	C, E, M	-	C, E, M	H, C	H, E, M	13	54
8. *Juniperus communis*	-	E, M	-	-	E, M	-	4	17
9. *Picea smithiana*	H, E	-	C, M	-	C	-	5	21
10. *Pinus wallichiana*	-	H	-	-	E, M	-	3	12
Total activity of extracts (%)	H=5 (50%) C=1 (10%) E=5(50%) M=3(30%)	H=3(30%) C=2(20%) E=5(50%) M=3(30%)	H=0(0%) C=2 (20%) E=0(0%) M=2(20%)	H=2(20%) C=2(20%) E=4(40%) M=2(20%)	H=1(10%) C=6(60%) E=5(50%) M=4(40%)	H=3(30%) C=1(10%) E=3(30%) M=2(20%)		

H- hexane extract, C- chloroform extract, E-ethanol extract, M-methanol extract

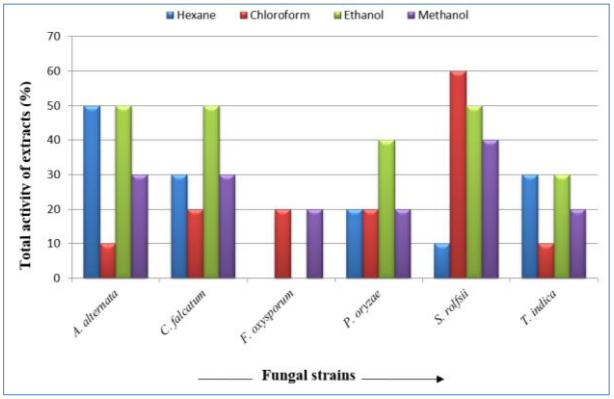

Figure 1: Antifungal potential of different extracts of gymnosperms against fungi

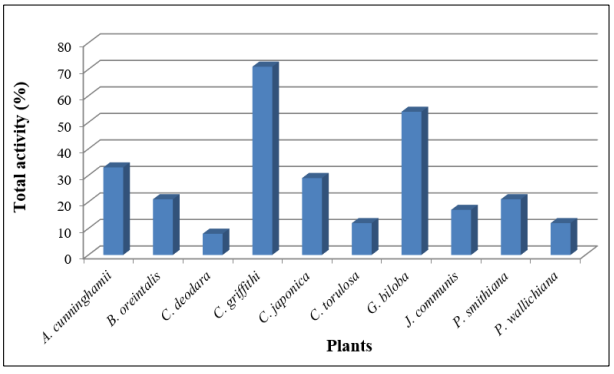

Figure 2: Total activity of tested gymnosperms

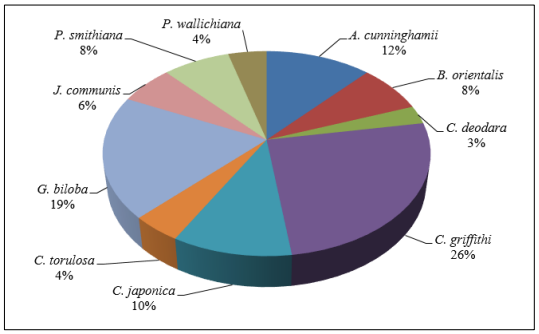

Figure 3: Relative antifungal activity of tested gymnosperms

DISCUSSION

The present study was carried out to exploration of gymnospermous medicinal plants from Nainital and its suburbs in Kumaun Himalaya (Uttarakhand), India based on the antimicrobial activity of selected plant extracts. The available literature shows that gymnospermic plants have not been adequately studied compared to angiosperms especially in Kumaun Himalayan region, Uttarakhand [34-37]. More over in regards to the bioactivity of Himalayan gymnosperms, a little information is available [38].

In this study 10 gymnospermous plants were examined for their antifungal activity by using polar and nonpolar solvents (methanol, ethanol, chloroform and hexane) extracts of their leaves occurring at high altitudes in Kumaun Himalaya, India against a wide range of pathogenic fungal organisms.

As evidence from the table 1 and 2, all the extracts of *C. griffithi* and *G. biloba* were performed a significant antifungal potentiality.

***Ginkgo biloba*:** Xie *et al.* [39] observed that the *G. biloba* leaf extract (GBE) was effective in inhibiting microbial growth whereas addition of sodium ethylenediaminetetraacetic acid (EDTA) enhances the antimicrobial activity of GBE against foodborne causing bacteria (*Listeria monocytogenes*). Haung *et al.* [40] isolated an antifungal protein termed as GAFP (ginkgo antifungal protein) from the leaves of *G. biloba*. This protein exhibited potent antifungal activity against *Pellicularia sasakii, A. alternata, F. graminarium* and *F. moniliforme.* But in this study *F. oxysporum* was resistant pathogen as no extract of *G. biloba* was able to inhibit this strain. In a study on antifungal activity of *G. biloba*, Baranowska and Wiwart [41] investigated antifungal activity of bioflavones isolated from leaves and found it is active against *A. alternata* at 200 µg/ml while *Cladosporium oxysporum* and *F. culmorum* at 100 µg/ml. But in our study, the range of MIC values of all tasted leaves extracts of *G. biloba* was observed 15.62 to 125 µg/ml for *A. alternata.*

Recently, Sati *et al.* [42] studied antifungal potential of leaf extracts of *G. biloba* through plate assays against four pathogenic fungi. They observed that methanolic extract was the best amongst the organic solvents used. Singh *et al.* [43] reported biologically active secondary metabolites groups, like terpenoids, polyphenols, organic acids, and

amino acids have been isolated from the *G. biloba* leaf extracts. Therefore, the synergistic effect of these compounds might be responsible for remarkable antifungal activity of *G. biloba* leaves extracts in our study.

***Cephalotaxus griffithi*:** It is clear from the table 1 and 2, that all the extracts of *C. griffithi* were showed highest fungitoxic activity. It has been reported that the flavonoids present in *Cephalotaxus* spp. were mainly responsible for such biological activities [44]. Kamil *et al*. [45] isolated and characterized six flavonoids. Recently, Moirangthem, *et al*. [46] studied antioxidant, antibacterial, cytotoxic, and apoptotic activity of stem bark extracts of *C. griffithi*. They found that the acetone and methanol extracts were most active but as evident from the table 2, hexane (non-polar) extract of *C. griffithi* performed lowest MIC value (7.81µg/ml) against *T. indica.*

***Araucaria cunninghamii*:** As evident from the available literature *A. cunninghamii* is well documented for its use for remedies of various ailments [33]. Relying upon the results obtained in the present investigation, it is clear that almost all extracts were active against the pathogenic fungi. Some phytochemicals and pharmacological studies for other species of this genus have been performed [47, 48, 49].

In a related study Caspedes *et al*. [47] reported antimicrobial activities of "Ligans" isolated from heartwood of *A. araucana* against various pathogenic bacteria and fungi. They observed that the methanol extract had the highest activity against pathogenic fungi *T. versicolor*, *Fusarium* spp. and *Trichophyton mentagrophytes*, inhibiting completely the growth. In our study, *F. oxysporum* was found to be the most resistant pathogen to all the extracts of *A. cunninghamii*. This may be because this fungal strain contains some active enzymes, which helps in metabolizing the secondary metabolites.

The results suggests that *A. cunninghamii* has broad spectrum antimicrobial activity, which may be due to the presence of various secondary metabolites like ligans, bioflavones, diterpenes, tannins, terpenoids, flavonoids, alkaloids, glycosides, phenols, steroids and sugar derivatives [38, 50].

***Biota orientalis*:** The results of the present study indicate that the plant *B. orientalis* assayed possess antifungal properties (Table 1). As evident from the literature *B. orientalis* is well documented for its use for remedies of various ailments [51, 52]. In folk medicine *B. orientalis* has been used to treat bronchial catarrh, cystitis, urine carcinomas, amenorrhea and rheumatism [53]. Essential oil of *B. oriantalis* has been widely used in steam bath. "THUJOIN" rich fraction separated from crude ethanolic extract of Biota had been reported to possess anticancer potential [54]. In the present investigation the ethanol and methanol extracts of *B. orientalis* showed a moderate antifungal activity against tested fungal strains (Table 1 and 2). However it is remarkable to note that ethanol and methanol extracts were found more potential than the standard antifungal agent "Clotrimazol" used in present study (Table 1). This might be due to the presence of α-pinene, α-cedrol, caryophyllene, limonene, α- ter-pinolene and α-terpinyl acetate, active compound in leaf extract of this plant [55]. Antifungal potential of *B. orientalis* was also evaluated by Guleria and Kumar [56] and Guleria *et al*. [55] but using their essential oils. Recently Srivastva *et al*. [57], reviewed biological properties of *B. orientalis* and concluded that *B. orientalis* has the great potentiality against a number of health problem.

***Cedrus deodara*:** It is interesting to note that methanol extract of *C. deodara* was found most active against *F. oxysporum*, performed 53% inhibition with MIC, 125 µg/ml. This might be due to that substances which are responsible for antifungal activity are more soluble in methanol than other solvent. This study supported by earlier studies made by Saller *et al*. [58] and Reichling [59]. They have found a range of antimicrobial active compounds in *C. deodara* like α & β pinene, Himachalol, Camphene, α & β-Himaanchalene, Cadinene, α Terpinenol.

Dikshit *et al*. [60] determined the fungicidal activity of *C. deodara* oil against 10 strains of fungi and found significant activity. Digrak *et al*. [61] also studied antifungal activity of leaves cones and bark of *C. libahi*, chloroform, actone and methanol extract against various pathogenic fungi. They found that methanol had the highest activity. It supports the present findings as methanol extract was found most effective fraction. The antifungal effects of essential oil of *C. deodara* as well as some of its active compounds against storage moulds have also been investigated by a few workers [62, 63].

***Cryptomeria japonica*:** Recently, SuYeon *et al*. [64] reported the antifungal activity of extracts of different parts of *C. japonica* against *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum gypseum.* They added that Black heartwood extract possesses the highest antifungal activity against dermatophytes. In the present investigation the four extracts of *C. japonica* leaves also tested against pathogenic fungal strains and found very significant findings in ethanol extract. In a study, Matsushita *et al*. [65] also determined antifungal activity of ethanol extract of sawdust of *C. japonica* heartwood and fractionated it with toluene and *n*-hexane. They found that the *n*-hexane-soluble fraction showed the most inhibition activity among the fractions against phytopathogenic microorganisms, namely *Fusarium oxysporum, Phytophthora capsici, Pythium splendens*, and *Ralstonia solanacearum*. Table 2 indicates that chloroform extract of *C. japonica* showed moderate inhibitory activity against *F. oxysporum* at 125µg/ml.

The majority of earlier investigators worked on the antifungal activity of wood part of *C. japonica*. Therefore, it is the first report on the antifungal activity of *C. japonica* against *A. alternata, C. falcatum, P. oryzae, S. rolfsii,* and *T. indica.*

***Cupressus torulosa*:** As evident from the table 1 and 2, the antifungal potentiality of *C. torulosa* is very good against all the tested microbes. Out of 4 solvents used for extraction of *C. torulosa* leaves extracts ethanol and chloroform extracts were found the most effected against all the test microbes. *In vitro* antifungal studies on *C. torulosa* cone extracts were also carried out by Sellappan *et al*. [66] using, *Candida albicans*, *Aspergillus flavus*, *Trichoderma lignorum* and *Cryptococcus neoformans* and found a significant antifungal activity of cone extract against all the pathogen tested. These activities may be attributed to the presences of the mono-, sesqui- and di-terpenes compounds in *C. torulosa* extracts [66, 67]. These chemical components probably exert their toxic effects against microorganisms through the disruption of bacteria or fungal membrane integrity since they are known to increase membrane permeability as well as the precursors of the resins that have been claimed to also contain the antifungal properties [68-70].

***Juniperus communis*:** Clark *et al*. [71] assayed hexane and methanol extract of heartwood, bark/sapwood and leaves of 12 taxa of

Juniperus including *J. communis* from the US for their antifungal activity. They found that methanol extract from the leaves of *J. osteosperma* and *J. califirnica* had highest inhibitory activity against *Trichophyton mentagrophytes* and hexane extract showed highest activity against *Cryptococcus neoformans*. Recently, Carbal *et al*. [72] studied essential oil of *J. communis* needles for antifungal activity of dermatophytes, yeast and *Aspergillus* sp. and found dermatophytes are more sensitive than yeast and *Aspergillus* sp. The antifungal activity of essential oil of *J. oxycedrus* leaves was also investigated against dermatophytic fungus *Candida* and *Aspergillus* [73]. Therefore it is the first report on the antifungal activity of Kumaun Himalayan species *J. communis* leaves extract against many new pathogenic fungal strains.

Nunez *et al*. [74] isolated three sesquiterpenes from the ethanolic extract of *J. lucayna* wood for their antifungal activity against *Botrytis cineria.* Wedge *et al.* [75] investigated chemical composition and antifungal activity of the essential oil of two Tibetan species of *J. saltuaria* and *J. squamata*. The essential oil of these species was found effective on the inactivation of *Colletotrichum aculatum*, *C. fragariae* and *C. gloeosporioides*. On the other hand, in present investigation ethanol and methanol extracts of *J. communis* leaves were performed a significant inhibitory activity against *C. falcatum* and *S. rolfsii* (Table1). Nunez *et al.* [74] suggested that presence of bioactive sesquiterpenes in *Juniperus* sp. are responsible for antifungal activity.

***Picea smithiana*:** Radulescu *et al.* [76] reported the positive antifungal activity of the volatile oil of *Picea* against *Candida* and *Aspergillus* sps. They suggested that *Picea* plant oil contains alpha-pinene, alpha-phellandrene, dipentene, bornylacetate, cadinene, S-guaiazulene and a bicyclic sesquiterpen compounds might be responsible for its antimicrobial potential.

The essential oils with high monoterpene hydrocarbon levels, such as pine oil, are found very active against fungi especially *Fusarium culmorum* [77]. Digrak *et al.* [61] investigated antifungal activity of chloroform, methanol and acetone extracts of leaves, resins, bark, cones and fruits of *P. brutia* and *P. nigra.* The methanol and acetone extract were found more effective against *Candida tropicalis*, *C. albicans* and *Penicillium italicum*.

***Pinus wallichiana*:** In the present study *P. wallichiana* was found less active compare to other gymnosperms tested. While an effective inhibition potential was observed in ethanol and methanol extract against *S. rolfsii* (MICs, 15.62 and 31.25µg/ml). Baranowska *et al*. [78] also investigated antifungal activity of the pine essential oil towards *Fusarium culmorum*, *F. solani* and *F. poae*. Recently, Lee *et al*. [79] investigated the antifungal activity of essential oil of *Pinus densiflora* against 8 pathogenic fungal strains and found its highest activity against *Cryptococcus neoformans* and *Candida glabrata*.

In this study two pathogenic fungi, *F. oxysporum* and *T. indica* were found resistant towards the most of the plant extracts tested (Table 1). This might be due to the fact that the plant pathogens are continuously evolving themselves against photochemicals and during this process they might have modified their metabolism or developed some resistant in their genetic element. The variation on fungitoxicity of the concerned plant extracts against phytopathogenic fungi may be due to considerable variations in their constituents and variation in fungal species itself [80].

The leaf extracts of *C. griffithi* (hexane extract) and *G. biloba* (ethanol extract) showed effective MIC values as compare to chemical fungicide (MIC 7.81 µg/ml). It supports the traditional use of these plant extracts as antiseptics [81, 82]. Plants do not contain an immune system but mechanisms to defend themselves from infection by a variety of pathogens might be due to synthesis of bioactive organic compounds by them [83] and antifungal proteins [84] and peptides [85].

Crude plant extracts are generally a mixture of active and non-active compounds, and MICs of less than 100µg/ml suggest good antimicrobial activity [86]. In this study, at many cases MICs values were found lower than 100µg/ml, demonstrating strong antifungal activity of extracts. The present findings are in agreement with the report of Banso *et al*. [87] who also observed that higher concentrations of antimicrobial substances showed more growth inhibition. In addition, the antimicrobial activity of plant extracts might not be due to the action of a single active compound, but the synergistic effect of several compounds that are in minor proportion in a plant [88]. This may be due to numerous compounds within the crude extracts interfering with the actions of one another. Once the crude extract was diluted, the inhibiting effect of one extract on the other was significantly reduced. This emphasizes the need to know the compound/s responsible for the inhibitory activity through studies to purify, identify and characterize the biomolecules.

Statistical analysis

On the basis of fungitoxic potential of selected plants four Hierarchical Clusters are made (Fig.4). As shown in fig. 4, methanol extracts of *A. cunninghamii, C. griffithi* and *G. biloba* grouped in same cluster which indicates that they performed closer inhibitory activity. As evident from the table 1, no effect was observed for methanol extracts of *C. griffithi* and *G. biloba* against *F. oxysporum*. Similarly *B. orientalis, C. japonica* and *C. torulosa* also found in same cluster but far away from *A. cunninghamii, C. griffithi* and *G. biloba* because no inhibition was found in methanol extracts of *B. orientalis, C. japonica* and *C. torulosa* against *T. indica*. Whereas, ethanol extracts of *C. griffithi, C. japonica* and *G. biloba* performed in same cluster and table 1 indicates that *F. oxysporum* fully resistant to these selected plant extracts. As evident from the table 1, all the tested ethanol extracts of listed plants showed a definite activity against *S. rolfsii* except ethanol extract of *C. deodara.* It is interesting to note that ethanol extract of *C. deodara* observed in different cluster among the tested plants extract.

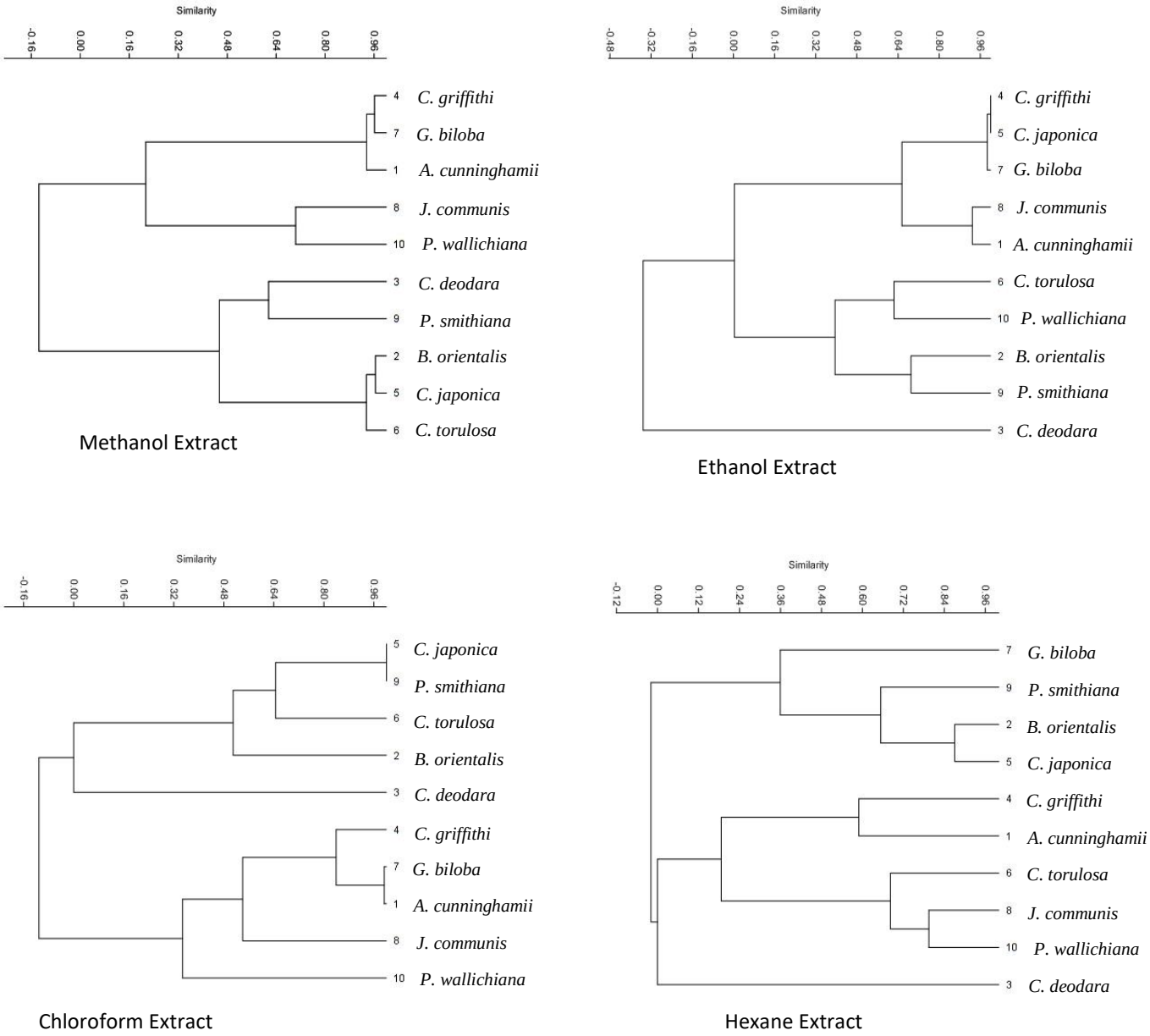

Figure 4: Hierarchical Clusters of four extracts of selected plants: **1-** *A. cunninghamii*, **2-***B. orientalis*, **3-***C. deodara*, **4-***C. griffithi*, **5-***C. japonica*, **6-** *C. torulosa*, **7-** *G. biloba*, **8-** *J. communis*, **9-** *P. smithiana*, **10-** *P. wallichiana*

The antifungal activity of chloroform extracts of *C. japonica* and *P. smithiana* comparatively same because chloroform extracts of both the plants have no effect against *C. falcatum*, *P. oryzae* and *T. indica* (Fig. 4). As well as chloroform extracts of *A. cunninghamii* and *G. biloba* also observed in same cluster due to their inactiveness against *F. oxysporum*. Moreover, chloroform extracts of both the plants (*A. cunninghamii* and *G. biloba*) showed their highest inhibitory effect against *S. rolfsii* (percent inhibition, 51 and 62, respectively). It is clear from the fig. 4, that chloroform extracts of *P. wallichiana* and *C. deodara* observed in separate cluster and table 1 also showed that chloroform extracts of *P. wallichiana* and *C. deodara* performed as a weak agent against all the tested pathogenic fungi.

On the other hand hexane extracts of all the tested plants showed the maximum variation against studied fungal pathogens. The effectiveness of particular compound against microorganism is also dependent on the metabolism of the organism as there are reports especially for fungi that the secondary metabolites of plant origin are metabolized by fungal enzymes [89].

The researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occurs causing cell death [90]. The present study indicates that application of plant extracts as biocontrol agents would be an effective measure in controlling plant diseases and gymnospermous plant extracts may be an attractive alternative for the use of natural product to control phytopathogenic fungi avoiding chemical fungicide.

It is also noteworthy that in many instances the test plant extracts showed their better antifungal activity (more than 50% inhibition)

than the commercially used antifungal drug Clotrimazole (inhibition range, 35-48%). Thus these findings suggest that the plants extracts contain more effective chemical compounds than the commercially available antibiotics to control various plant and animal diseases.

A perusal of available literature shows that the most of the earlier investigators worked on the antifungal activity against dermatophytes, yeast species. Therefore, this is the first report on antifungal activity of gymnosperms against *A. alternata, C. falcatum, P. oryzae, S. rolfsii* and *T. indica*.

Sati *et al*. [51] suggested that in Kumaun Himalaya a few workers carried out the bioactivity of angiospermic plants but there is complete dearth of information regarding the antimicrobial activity of gymnosperm plants. Therefore, this study on antimicrobial activity of gymnosperms of Kumaun Himalaya is one step to bridge the gap in this direction.

Based on statistical analysis although, it is difficult to correlate the antimicrobial activity to single compound or classes of compounds as it might be possible that the antifungal effect, the result of many compounds acting synergistically [91], but the present work would be extremely useful to determine the utility of gymnospermic plant/parts extracts in fungal disease management.

CONCLUSSION

The results obtained from this work showed that plant extracts of Kumaun Himalayan gymnospermous plants screened exhibit antifungal effects against six plant pathogenic fungi (*A. alternata, C. falcatum, F. oxysporum, P. oryzae, S. rolfsii* and *T. indica*). In particular, leaves extracts of *Cephalotaxus griffithi*, and *Ginkgo biloba* suggest effective bioactive compounds for growth inhibition of the fungi. Even at low concentrations, these species showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. The high proportion of active extracts in the assayed species, selected according to available ethnobotanical data, corroborates the validity of this approach for the selection of plant species in the search for a specific activity. Isolation and identification of active compounds in the plant extract that responsible for antifungal activity is needed, in order to assess the efficacy, mode of action and possible side effects of their use. In addition, formula development is important step to get economical and effective use of plant extract as fungal diseases control agent. Therefore the results of this study on the evaluation of antifungal potential of gymnosperms not only important to supports the traditional uses of these plants but also useful to generate additional data on crop protection potential of medicinal plants of Kumaun Himalaya needed for new fungicide discoveries.

Acknowledgements

We are thankful to University Grant Commission, New Delhi for financial support from UGC-BSR under SAP scheme. The authors wish to thank Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar for providing fungal cultures.

REFERENCES

1. Raj DS, Vennila JJ, Aiyavu C, Panneerselvam K. The hepatoprotective effect of alcoholic extract of *Annona squamosa* leaves on experimentally induced liver injury in Swiss albino mice. International Journal of Integrative Biology. 2009; (3):182-186.
2. Cutler HG, Cutler SJ. Biological active natural product: Agrochemical CRS Press, Boca Raton: USA: 299, 1999.
3. Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris* L.essential oil as a safe botanicalpreservative against post-harvestfungal infestation of foodcommodities. Food Science. Emerg. 2008; 4:575-580.
4. Sener B, Bingol F, Erdogan I, Bowers WS, Evans PH. Biological activity of some Turkish medicinal plants. Pure Appl. Chem. 1998; 70:403-406.
5. Cardwell DA, Bradley AD, Hari Babu N, Kambara M, Lo W. Processing, microstructure and characterisation of artificial joins in top seeded melt grown Y-Ba-Cu-O. Supercond. Sci. Technol. 2002; 15:639.
6. Agrios GN. Losses caused by plant diseases. Plant Pathology. Elsevier, Oxford, UK. 2004; p.29-45.
7. Harris CA, Renfrew MJ, Woolridge MW. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contamination, 2001; 18:1124-1129.
8. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective Nationwide Surveillance Study. Clin. Infect. Dis. 2004; 39:309-317.
9. Venkatesan P, Perfect JR, Myers S. Evaluation and management of fungal infections in immune compromised patients. Dermatol. Ther. 2005; 18:44-57.
10. Galvan B, Mariscal F. Epidemiología de la candidemiaem UCI. Rev. Iberoam. Micol. 2006; 23:12-15.
11. Cruz MCS, Santos PO, Barbosa Jr AM, De Melo DL, Alviano CS, Antoniolli AR, *et al*. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J. Ethnopharmacol. 2007; 111:409-412.
12. Souza ACM, Kato L, Silva CC, Cidade AF, Oliveira CAM, Silva MRR. Antimicrobial activity of *Hymenae amartiana* towards dermatophytes and *Cryptococcus neoformans.* Mycoses. 2010; 53:500-503.
13. Kim YM, Lee CH, Kim HG, Lee HS. Anthraquinones isolated from *Cassia tora Leguminosae* seed show an antifungal property against phytopathogenic fungi. J. Agric. Food Chem. 2004; 52:6096-6100.
14. Yang X, Ma X, Yang L, Yu D, Qian Y, Ni H. Efficacy of *Rheum officinale* liquid formulation on cucumber powdery mildew. Biol. Cont. 2010; 522:167-173.
15. Kumar P, Joshi S, Sati SC, Rai D. A comparative evaluation of phytochemical and antibacterial properties of *Ricinus communis* Linn and *Thevetia peruviana* Schum. Of Kumaun Himalaya. Mintage Journal of Pharmaceutical and Medical Sciences. 2016; 5(2):13-19.
16. Kubo M, Kimura Y, Shin H, Haneda T, Tani T, Namba K. Studies on the antifungal substance of crude drug (II) on the roots of *Polygonum cupsidatum* Sieb. Et Zucc (Polygonaceae). Sho- yakugaku Zasshi, 1981; 35:58-61.
17. Vuuren SFV, Naidoo D. An antimicrobialinvestigation of plants used traditionally in southern Africato treat sexually transmitted infections. J. Ethnopharmacol. 2010; 130:552-558.
18. Bhengraj AR, Dar SA, Talwar GP, Mittal A. Potential of a novel polyherbal formulation BASANT for prevention of *Chlamydia trachomatis* infection. Int. J. Antimicrob. Agents, 2008; 32:84-88.
19. Motsei ML, Lindsey KL, Van Staden J, Jager AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. J Ethnopharmacol. 2003; 86(2-3):235-241.
20. Bryan-Brown T. The pharmacological action of Taxine. Quat. J. pharma. Pharmacol. 1932; 5:205-219.
21. Appendino G. Taxol (paclitaxel): Historical and ecological aspects. Fitoterapia. 1993; 64:5-25.
22. Kumar P, Sati SC. Phenology of Gymnosperms of Nainital, Kumaun Himalaya, India. The International Journal of Plant Reproductive Biology. 2016; 8(2):145-157.

23. Singh V. Traditional remedies to treat asthma in northwest and trans-Himalayan region in Jammu and Kashmir state. Fitoterapia. 1995; 66:507-509.
24. Erdemoglu N. Sener B. Antimicrobial activity of heartwood of *Taxus baccata*. Fitoterapia. 2001; 72:59-61.
25. Sati SC, Joshi S. Antimicrobial potential of leaf extracts of *Juniperus communis* L. from Kumaun Himalaya. Afr. J. Microbiol. Res. 2010; 4:1291-1294.
26. Sati SC, Kumar P. Assessment of Himalayan juniper, *Juniperus squamata* buch– ham ex d. don for phytochemical screening and antimicrobial potential against some infection causing pathogens. World Journal of Pharmaceutical Research. 2015; 4(10):998-1011.
27. Niemann GJ. Distribution and evolution of the flavonoids in gymnosperms. In: The Flavonoids. Advances in research since 1980 (Harborne J.B., ed). Chapman and hall, London, 1988; pp 469-478.
28. Harborne JB. Baxter W. the chemical dictionary of economic plants. Wiley and Sons, Chichester, 2001; p.582.
29. Parmar VS, Jha A, Bisht KS, Taneja P, Singh SK, Kumar A, *et al*. Constituents of Yew trees. Phytochemistry.1999; 50:1267-1304.
30. Balandrin MF, Kjocke AJ, Wurtele E. Natural plant chemicals: sources of industrial and mechanical materials. Science, 1985; 228:1154-1160.
31. Song W, Wang HJ, Bucheli P, Zhang PF, Wei DZ, Lu YH. Phytochemical profiles of different mulberry (Morus sp.) species from China. J Agric Food Chem. 2009; 57:9133-9140.
32. Grover RK, Moore JD. Toximetric studies of fungicides against brown rot organism. *Sclerotina fruticola*. Phytopathology. 1962; 52:876-880.
33. Sati SC, Joshi S. Antifungal potential of *Araucaria cunninghamii* Sweet. (Araucariaceae) plant extract. Int. J. Pharmacology and Toxicology Sciences. 2013; 3(1):15-21.
34. Joshi S, Sati SC. Antifungal Potential of Gymnosperms: AReview. In "*Microbes: Diversity and Biotechnology*" (Eds. S. C. Sati and M. Belwal). 2012; pp 333-345.
35. Sati SC, Kumar P, Joshi S. The bark extracts of Himalayan gymnosperm *Picea smithiana* (Wall.): A natural sources of antibacterial and antioxidant agent. International Journal of Pharmacy. Photon. 2015a; 106:445-452.
36. Sati SC, Takuli P, Kumar P, Khulbe K. Antibacterial activity of three medicinal plants of Kumaun Himalaya against some pathogenic bacteria. International Journal of Pharma Sciences and Research. 2015b; 6(11):1361-1368.
37. Joshi S, Sati SC, Kumar P. Antibacterial potential and ethnomedical relevance of Kumaun Himalayan Gymnosperms. The Journal of Phytopharmacology. 2016; 5(5):190-200.
38. Sati SC, Joshi S. Aspects of antifungal potential of ethnobotanically known medicinal plants. Res J Med Plants. 2011; 5(4):377-391.
39. Xie L, Hettiarachchy NS, Jane ME, Johnson MG. Antimicrobial activity of *Ginkgo biloba* leaf extract on *Listeria monocytogenes*. Journal of Food Science. 2003; 68(1):268-270.
40. Haung X, Xie W, Gong Z. Characterization and antifungal activity of a chitin binding protein from *Ginkgo biloba*. FEBS letters. 2000; 478:123-126.
41. Baranowska MK, Wiwart M. Antifungal Activity of Biflavones from *Taxus baccata* and *Ginkgo biloba*. Z. Naturforsch. 2003; 58c:65-69.
42. Sati P, Pandey A, Palni LMS. Antimicrobial potential of leaf extracts of *Ginkgo biloba* L., growing in Uttarakhand, India. National Academy Science Letters. 2012; 35(3):201-206.
43. Singh B, Kaur P, Gopichand, Singh RD, Ahuja PS. Biology and chemistry of *Ginkgo biloba*. Fitoterapia. 2008; 79(6):401-418.
44. Bae K, Jin W, Thoung PT, Min BS, Na M, Lee YM, *et al*. A new flavonoid glycoside from the leaf of *Cephalotaxus koreana*. Fitoterapia. 2007; 78:409-413.
45. Kamil M, Khan NA, Ahmad I, Iiyas M, Rahman W. Chemical constituents of *Cephalotaxus griffithii*. J Indian Chem Soc. 1982; 59:1199.
46. Moirangthem DS, Talukdar NC, Kasoju N, Bora U. Antioxidant, antibacterial, cytotoxic, and apoptotic activity of stem bark extracts of *Cephalotaxus griffithii* Hook. f. BMC Complementary and Alternative Medicine. 2012; doi: 10.1186/1472-6882-12-30.
47. Cespedesa Carlos L, Avila JG, Garcıa AM. Antifungal and Antibacterial Activities of *Araucaria araucana* (Mol.) K. Koch Heartwood Lignans. Z.Naturforsch. 2006; 61c:35-43.
48. Mota MR, Criddle DN, Alencar NM. Modulation of acute inflammation by a chitin-binding lectin from *Araucaria angustifolia* seeds via mast cells. Naunyn-Schmiedeberg's Archives of Pharmacology 2006; 374:1-10.
49. Santi-Gadelha T, Gadelha CAA, Aragao KS, Oliveira CC. Purification and biological effects of *Araucaria angustifolia* (Araucariaceae) seed lectin. Biochemical and Biophysical Research Communications, 2006; 350:1050-1055.
50. Freitas AM, Almeida MTR, Andrighetti-Frohner CR. Antiviral activity-guided fractionation from *Araucaria angustifolia*leaves extract. J. Ethnopharmacol. 2009; 126:512-517.
51. Sati SC, Joshi S, Kumar P. Antibacterial activity of Kumaun Himalayan *Biota orientalis* L. Leaf extracts. African Journal of Microbiology Research. 2014; 8(6):603-608.
52. Joshi S, Sati SC, Kumar P. Efficacy of Kumaun Himalayan *Biota orientalis* Endl. leaves extracts against pathogenic fungi. International Journal of Pharmacology and Toxicology. 2016; 4(2):224-227.
53. Peng D, Wang XQ. Reticulate evolution in *Thuja* inferred from multiple gene sequences: implications for the study of biogeographical disjunction between eastern Asia and North America. Mol Phylogenet Evol, 2008; 47:1190-1202.
54. Biswas R, Mandal SK, Dutta S, Bhattacharyya SS, Boujedaini N, Khuda-Bukhsh AR.Thujone-Rich Fraction of *Thuja occidentalis* Demonstrates Major Anti-Cancer Potentials: Evidences from In Vitro Studies on A375 Cells. Evid Based Comp Alt Med, 2011; 568148.
55. Guleria S, Kumar A, Tiku AK. Chemical Composition and Fungitoxic Activity of Essential Oil of *Thuja orientalis* L. Grown in the North-Western Himalaya. Z. Naturforsch, 2008; 63c:211-214.
56. Guleria S, Kumar A. Antifungal activity of some Himalayan medicinal plants using direct bioautography. Journal of Cell and Molecular Biology, 2006; 5:95-98.
57. Srivastava P, Kumar P, Singh DK, Singh VK. Biological Properties of *Thuja orientalis* Linn. Advances in Life Sciences. 2012; 2:17-20.
58. Saller R, Reichling J, Hellenbrecht D. Phytotherapie-Klinische, Pharmakologische Und Pharmazeutische Grundlagen. Haug-Verlag, Heidelberg.1995.
59. Reichling J. Australian and New Zealand tea tree oil as the source of noval antiinfective agents. In: Wildi, E., M. Wink (eds.), Trends in Medicinal Plant Research, pp: 69–80. Romneya-Verlag, Dossenheim.2001.
60. Dikshit A, Dubey NK, Tripathi NN. Dixit SN. Cedrus oil- A promising storage fungitoxicant. Journal of Stored Products Research. 1983; 19(4): 159-162.
61. Digrak M, Iicim M, Alwa NH. Antimicrobial activities of several parts of *Pinus brutia, Juniperus onycedrus, Abies cilicia* and *Pinus nigra*. Phytotherapy Res. 1999; 13(7):584-587.
62. Essien EP, Essien JP. Control of fungal deterioration of two varieties of *Capsicum annuum*during storage by the essential oil of *Cedrus deodara.* Nig J Natl Pdts Med. 2000; 4:62-64.
63. Parveen R, Ahmed M, Azmi RM, Tariq SM, Mazhar H, Shaukat M, Naqvi SNH. Determination of antifungal activity of *Cedrus deodara* root oil and its compounds against *Candida albicans* and *Aspergillus fumigates.* Pak. J. Bot.2010; 42(5):3645-3649.
64. Su-Yeon Lee, Gwak Ki-Seob Lee, Jun-J Yeo, Hwan-Myeong Choi, In-Gyu. Antifungal Activity of Extracts from Black Heartwood of *Cryptomeria japonica* against Dermatophytes. Journal of the Korean Wood Science and Technology. 2009; 37(3):265-273.
65. Matsushita Y, Hwang Y, Sugamoto K, Matsu T. Antimicrobial activity of heart wood components of sugi (*Cryptomeria japonica*) against several fungi and bacteria. Journal of Wood Science, 2006; 52:552-556.
66. Sellappan M, Palanisamy D, Joghee N, Bhojraj S. Chemical Composition and Antimicrobial Activity of the Volatile Oil of the Cones of *Cupressus torulosa* D. Don from Nilgiris, India. Asian Journal of Traditional Medicines, 2007; 2(6):206-211.
67. Lohani H, Gwari G, Andola HC, Bhandari U, Chauhan N. α-pinene rich volatile constituents of *Cupressus torulosa* D. don from Uttarakhand Himalaya. Indian J Pharm Sci. 2012; 74:278-80.

68. Uribe S, Ramirez T. Effects of β-pinene on yeast membrane functions. J Bacteriol. 1998; 161:195-200.
69. Knoblock K, Pauli A, Iberi B, Weis N, Weigand H. Antibacterial activity and antifungal properties of essential oil components. J Essent Oil Res. 1998; 1:119-128.
70. Filipowiez N, Kaminski M, Kurlenda J, Asztemborska M. Antibacterial and antifungal activity of Juniper Berry oil and its selected components. Phytother Res. 2003; 17:227-231.
71. Clark MA, McChesney JD, Adams RP. Antimicrobial properties of heartwood, bark/sapwood and leaves of *Juniperus* species. Phytotherepy Research. 1990; 4(1):15-19.
72. Cabral C, Francisco V, Cavaleiro C, Gonçalves MJ, Cruz MT, Sales F, Batista MT, Salgueiro L. Essential oil of *Juniperus communis* subsp. alpina (Suter) Celak needles: chemical composition, antifungal activity and cytotoxicity. Phytother Res. 2012; 26(9):1352-1357.
73. Cavaleiro C, Pinto E, Gonçalves MJ, Salgueiro L. Antifungal activity of *Juniperus* essential oils against dermatophyte, *Aspergillus* and *Candida* strains. Journal of Applied Microbiology. 2006; 100(6):1333-1338.
74. Nunez YO, Salabarria IS, Collado IG, Hernandez-Galan R. Sesquiterpenes from the wood of *Juniperus lucayana*. Phytochemistry. 2007; 68:2409-2414.
75. Wedge DN, Tabanca B, Sampson C, Werle B, Demirci K, Baser N, Peng D, Zhi Jun Jia L. Antifungal and insecticidal activity of two *Juniperus* essential oils. Nat. Prod. Comm. 2009; 4:123-127.
76. Radulescu V, Saviuc C, Chifiriuc C, Opra E, Carolina D, Marutescu L, Lazer V. Chemical composition and antimicrobial activity of essential oil from shoots Spruce (*Picea abies* L). Rev. Chim. (Buchaust). 2011; 62(1): 69-72.
77. Lis-Balchin M, Deans SG. Eaglesham E. Stermitz FR, Tawara JN, Boeckl M, Pomeroy M.Relationship between bioactivity and chemical composition of commercial essential oils. Flavour Fragr. J. 1998; 13:98-104.
78. Baranowska MK, Mardarowicz M, Wiwart M, Pobłocka L, Dynowska M. Antifungal Activity of the Essential Oils from Some Species of the Genus *Pinus*. Z. Naturforsch. 2002; 57c:478-482.
79. Lee JH, Lee, BK, Kim JH, Lee SH, Hong SK. Comparison of chemical compositions and antimicrobial activities of essential oils from three Conifer Trees; *Pinus densiflora*, *Cryptomeria japonica*, and *Chamaecyparis obtusa*. J. Microbiol. Biotechnol. 2009; 19(4):391-396.
80. Manoranjitham SK, Prakasam V, Rajappan K. Biocontrol of damping-off of tomato caused by *Pythium aphanidermatum*. Ind. Phytopathol. 2001; 54:59-61.
81. Alonso PE. Investigación sobre uso popular de plantas medicinales en la zona de Pedernal. 28 p.Centro Uruguayo de Tecnologí as Aplicadas CEUTA, Montevideo, Uruguay. 2000.
82. Heinzen H, Vazquez A, Dellacassa E, Villamil J, Bittencourt YG. The production and marketing of phytotherapics. p. 52-131. Fundaquim, Red Propymes, Uru. Tec., Fundasol, Cooperación Tecnica Alemana (eds.) Contributions for the development of the sector of medicinal and aromatic plants in Uruguay. Artes Gráficas, Montevideo, Uruguay.2004
83. Morrisey JP, Osbourn A. Fungal resistanceto plant antibiotics as a mechanism of pathogenesis.Microbiology and Molecular Biology Reviews.1999; 63:708-724.
84. Selitrennikoff CP. Antifungal proteins. Appliedand Environmental Microbiology 2001; 67:2883-2894.
85. Broekaert WF, Cammue BPA, De Bolle MFC, Thevissen K, De Samblanx GW, Osborn RW. Antimicrobial peptides from plants. Critical Reviews in Plant Sciences 1997; 16:297-323.
86. Webster D, Taschereau P, Belland RJ, Sand C, Rennie RP. Antifungal activity of medicinalplant extracts; preliminary screening studies. J. Ethnopharmacol. 2008; 115:140-146.
87. Banso A, Adeyemo SO, Jeremiah P. Antimicrobial properties of *Vernonia amygdalina* extract. Journal of Applied Science and Management.1999; 3:9-11.
88. Davicino R, Mattar MA, Casali YA, Graciela S, Margarita E, Micalizzi B. Antifungal activity of plant extracts used in folk medicine in Argentina. Revista Peruana de Biología. 2007; 14:247-251.
89. Anderson JJ and Dagley S. Catabolism of aromatic acids in *Tricospa cutaneum*. J Bacteriol. 1980; 141(2):534-543.
90. Burt S. Essential oils: their antibacterial properties and potential application in foods: a review, Int. J. Food Microbiol., 2004; 94:223-253.
91. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol. 2004; 21:33-42.

HOW TO CITE THIS ARTICLE

Joshi S, Kumar P, Pant P, Sati SC. A comparative evaluation of Kumaun Himalayan Gymnosperms for their Antifungal potential against plant pathogenic fungi. J Phytopharmacol 2018; 7(3):230-241.