IN VITRO ANTIFUNGAL ACTIVITY OF SOME MEDICINAL PLANTS FROM NARAYANPUR, BASTAR, CHHATTISGARH AGAINST *ASPERGILLUS NIGER*

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ABSTRACT

Chhattisgarh state is declared as "Herbal State" with its bountiful natural resources of forests and agricultural fields and it is equally well known for its medicinal Hot spots. The dense forests have rich and unique biological diversity with a wide range of medicinal plants. Narayanpur district the southern parts of Chhattisgarh state is the store houses of precious medicinal plants and provide the local people, mostly tribes with livelihood opportunities. In the present study in vitro antifungal activity of petroleum ether, chloroform, acetone and aqueous leaves extracts of four plants (Desmodium gangeticum (L.) DC., Celastrus peniculatus Willd., Dillenia indica Linn., Sterculia urens Roxb.) and tuber extracts of one plant (Dioscore hispida Dennst.) was determined against Aspergillus niger. Antifungal activity was performed by using Poison food technique on Potato dextrose agar media (PDA). It was observed that tuber of D. hispida in aqueous extract showed maximum growth inhibition % at 1000µl conc. on A. niger (92 ± 0) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77) followed by D. gangeticum in acetone leaf extract showed maximum growth inhibition % at 1000µl conc. on A. niger (91.55 ± 0.44) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77), C. peniculatus in chloroform leaf extract showed maximum growth inhibition % at 1000µl conc. on A. niger (86.66 ± 4.07) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77), S. urens in acetone leaf extract showed maximum growth inhibition % at 1000µl conc. on A. niger (86.22 ± 2.70) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77), D. indica in chloroform leaf extract showed maximum growth inhibition % at 1000µl conc. on A. niger (85.77 ± 4.88) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77).

Keywords: *Medicinal plants, antifungal activity, growth inhibition percentage (%), SPSS analysis.*

1. INTRODUCTION

Despite tremendous success in development of medical sciences in recent years, infectious diseases remain the second leading cause of human death worldwide (WHO 2002). Fungal infections have contributed to the increase of life-threatening systemic fungal infections in recent years (Perea and Patterson 2002). Some of the factors responsible for these are: the expansion of severely ill and/or immunocompromised patient population, including HIV-infected patients, patients with cancer who have chemotherapy-induced neutropenia, and transplant recipients who are receiving immunosuppressive therapy (Beck-Sague *et al.,* 1993; Perea and Patterson 2002). Individuals with weakened immune system are more susceptible to fungal infections. Fungal infections are major concerns in India with an increasing numbers of new reports from superficial to deep hospital-acquired infections every year. Although there are no comprehensive data on the real incidence of fungal infections, especially systemic ones in India, about 50 % of suspected individuals referred to our laboratory (Mycology Department of the Pasteur Institute of India) were found. Some Aspergillus species cause serious disease in human being, animals and plants.

Aspergillus niger is commonly regarded as a pathogenic allergen generally associated with lung infections in individuals with weak immune system. Because the conidia and conidiophores are small, readily air borne, can easily breathed in and cause deep or systemic mycosis (Kierownik., 1990). It usually affects superficial tissues such as impaired skin, nose, or ear ducts. Local lesions in external and middle ear, as well as in post operative cavities, can creat favorable conditions for fungal growth and subsequent otomysis (Kaur *et al.*, 2000; Kurnatowski and Kilipiak., 2001). It is also the causative agent of 'black mould' on the outside. The host range includes 37 genera of fruits and vegetables such as tomatoes, peanuts, grapes, onions, and mangoes and thus making *Aspergillus niger* a food 'spoilage' organism. The fungus is commonly found in the soil (Samson *et al.*, 2001).

In reference to medicinal plants Chhattisgarh state is declared as "Herbal State" with its bountiful natural resources of forests and agricultural fields and it is equally well known for its medicinal Hot spots. The dense forests which account 41.8 percent of the total geographical area. Chhattisgarh is significantly in endemism with respect to many herbs, shrubs and tree species having timber spices, fodder and medicinal values due to its diversified topography and variable climatic condition. Chhattisgarh State Medicinal Plants Board, in its survey, had identified occurrence of around 2,021 total number of plant species included medicinal and aromatic plants (MAP's) in the state. According to the State Forest Department, the production of Medicinal and Aromatic plants is about 87,065 metrictonne in the state (www.dailypioneer.com). Medicinal and aromatic plants (MAPs), and their derivatives appear to be a boon bestowed by nature on the mankind for prevention as well as cure of human health problems (diseases and disorders) since immemorial.

Previous study suggests that the antimicrobial compounds produced by plants and herbs are active against plants and human pathogenic microorganisms (Ramezani *et al.*, 2002). Current control of phytopathogenic agents is done using synthetic fungicides. Along with increasing of restriction regarding their use due to adverse effects on human and environments (Harris *et al.*, 2001) which appeared the need to find alternatives to fungicides, insecticides and pests. Therefore, interest in plant- derived drugs has been increasing, mainly due to the current extensive belief that "green medicine" is safer and more dependable than costly synthetic drugs (Parekh and Chanda., 2006). The demand on plant based therapeutics is increasing in both developing and developed countries due to the growing recognition that they are natural products, being non-narcotic having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor. In the present work, antifungal activity susceptibility test against *Aspergillus niger* was performed in different solvent extracts of medicinally important plants.

2. MATERIALS AND METHODS

Collection of plant sample

Mature green and disease free leaves of *Desmodium gangeticum* (L.) DC. (Fabaceae), *Celastrus peniculatus* Willd. (Celastraceae), *Dillenia indica* Linn. (Dilleniaceae), *Sterculia urens* Roxb. (Sterculiaceae) and tuber extracts of one plant *Dioscore hispida* Dennst. (Dioscoreaceae) were collected from Herbal Garden, Bakhrupara Kasthagar and tropical dry deciduous forest area of Narayanpur District during their flowering seasons (year: 2015 - 2016). Plants were identified by Vaidraj Ratan Dhar Department of Naturopathy and Yogic Science (Govt. Regd. No - MAH/394/06/THA). Photoplate no. 1

Preparation of crude extract

Leaves and tubers were washed 2-3 times with running tap water and rinsed twice with distilled water. Air dried under shade at room temperature for 1-2 weeks till the leaves becomes brittle enough to break easily and tubers were cut into small pieces and shade dried for 3-4 weeks until all water molecule evaporated. After complete drying plant parts were crushed to fine homogenous powder using mechanical grinder and transferred into air tight poly bags with proper labeling and stored at room temperature. Extraction was carried out by Soxhlet procedure as described by Hozowitz., 1984. Coarse powders of *D. gangeticum* (L.) DC. were subjected to solvent extraction method at a temperature not exceeding their boiling point. Twenty – five grams of powder was uniformly packed into a thimble prepared from Whatman No. 1 filter paper. Extraction was carried out for both aqueous and organic solvent (*viz.*, petroleum ether, chloroform, acetone) of increasing polarity. 250 ml of extracting solvent in flask is heated, and its vapours condense in condenser. The process of extraction was continued untill the solvent in thimble tube of an extractor become colorless. After that the solvent extracts were filtered with the help of Whatman's No.1 filter paper (Yadav and Agrawala., 2011). After filtration, the beaker is kept in waterbath and heated at 40-50°C till all the solvent got evaporated and extract was referred to as the crude extract (Muthukrishnan and Venkatachalan., 2012). Crude extracts was kept in refrigerator at 4°C for further use.

Antifungal activity susceptibility test:

Antifungal activity test against the pathogen was determined by Poison food technique (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapliyal, 2000). Potato dextrose agar (PDA) was used as a culture medium. From this required different concentration 100µl, 250µl, 500µl, 1000µl of extract was taken by sterilized pipette and mixed with 15 ml of cooled (45°C) molten PDA medium, poured into the sterile pertiplates and mixed well and allowed to solidify at room temperature for thirty minutes. The plates were left overnight at room temperature to check for any contamination to appear. Inoculation was done at the center of each plate with 5 mm mycelium block for each fungus. The mycelium block was prepared with the help of cork borer from the growing area of a 5 days old fungal culture. The fungal disc was transferred aseptically at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. Proper control (PDA without extract) serves as control. Antibiotics Amphotericine – B were used as positive control respectively (Mc Cutcheon *et al.,* 1994). The inoculated plates were incubated for 72 hours at temperature 26±1°C. Diameter of fungal Colonies is recorded by measuring the two opposite circumference of the colony growth. The average of triplicate of measurements was taken as colony diameter of the fungus in millimeters. To ensure the sensitivity of pathogens to the plant extract. The percentage inhibition of mycelial growth of the test fungus was calculated by the following formula

I (%) = (C - T/ C) x 100

Where, I = Percentage of inhibition, C = Diameter of fungal colony in control (mm),
T = Diameter of fungal colony in treatment (mm) (Rahman *et al.,* 2011).

STATISTICAL ANALYSIS:

Data analysis was done using SPSS statistical package for Windows (version 16.0, SPSS Inc). Data values were expressed as mean ± standard error. Statistical analysis was performed using One-way ANOVA, DMRT test using Duncan's univarent test was used for calculating for 5% level of significance ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

RESULTS

Results summarized in Table- 1. Antifungal activity in acetone leaf extracts of *D. gangeticum* showed maximum growth inhibition % at 1000µl conc. on *A. niger* (91.55 ± 0.44) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77). While the other chloroform, aqueous and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (83.55 ± 1.17) at lower concentration 100µl in acetone leaf extract. One-way ANOVA summary (F= 19.79; df= 19,40; $p \leq 0.05$) thus, the value showed significant difference is graphically represented in figure no.1. (Photoplate- 2)

In chloroform leaf extracts, *C. peniculatus* showed maximum growth inhibition % at 1000µl conc. on *A. niger* (86.66 ± 4.07) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77). While with petroleum ether, aqueous and acetone extract showed moderate sensitivity. Minimum growth inhibition % was recorded (80.89 ± 7.11) at lower concentration 100µl in chloroform leaf extract. One-way ANOVA summary (F= 2.727; df= 19,40; $p \leq 0.05$) thus, the values showed significant difference is graphically represented in figure no. 2.

In chloroform leaf extracts, *D. indica* showed maximum growth inhibition % at 1000µl conc. on *A. niger* (85.77 ± 4.88) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77). While with acetone, aqueous and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (82.66 ± 4) at lower concentration 100µl in chloroform leaf extract. One-way ANOVA summary (F= 7.26; df= 19,40; $p \leq 0.05$) reported significant difference values graphically represented in figure no.3.

In acetone leaf extracts, *S. urens* showed maximum growth inhibition % at 1000µl conc. on *A. niger* (86.22 ± 2.70) which was nearest to the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77). While with petroleum ether, chloroform and aqueous extract showed moderate sensitivity. Minimum growth inhibition % (76.89 ± 5.40) was recorded at lower concentration 100µl in acetone leaf extract. One-way ANOVA summary (F= 5.61; df= 19,40; $p \leq 0.05$) reported significant difference values graphically represented in figure no. 4.

In aqueous tuber extracts, *D. hispida* showed maximum growth inhibition % at 1000µl conc. on *A. niger* (92 ± 0) which was more than the value of the standard antibiotics Amphotericine- B (87.55 ± 1.77). While with chloroform, acetone and petroleum ether extract showed moderate sensitivity. Minimum growth inhibition % was recorded (89.77 ± 0.44) at lower concentration 100µl in aqueous tuber extract. One-way ANOVA summary (F= 9.354; df= 19,40; $p \leq 0.05$) reported significant difference values graphically represented in figure no. 5.

DISCUSSION

This study revealed that the selected medicinal plant extracts had antifungal activity potential against *A. niger*. Both organic solvent and aqueous extracts assayed indicated antifungal activity with varied degree depending on concentration and plant species. At 1000µl conc. aqueous tuber extract of *D. hispida* showed maximum growth inhibition % on *A. niger* followed by acetone leaf extract of *D. gangeticum*, chloroform leaf extract of *C. peniculatus*, acetone leaf extract of *S. urens* and chloroform leaf extract of *D. indica* respectively with significant difference of ($p \leq 0.05$) was reported when compared with the standard antibiotics Amphotericine- B. Similar work was performed by Avasthi *et al.,* (2010) using eight plant species, results revealed that five plant showed significant antifungal activity against *Aspergillus niger* the test pathogen by Poison food technique. *Syzygium aromaticum* and *Allium sativum* showed 100% inhibition of mycelia growth at 20% concentration. In *Cinnamomum zeylanicum*, *Trachy spermumammi* and *Piper nigrum* a moderate inhibition (52.4%, 48.93%, 46.2%) was reported whereas, no inhibition was in other plant products. Gautam *et al.,* (2015) reported that the essential oil of *Althaea officinalis* Linn. seed extracts exhibits percentage inhibition of 41.28% against *A. niger*, aqueous extracts with 36.27% and methanolic extracts with 23.89% of inhibition respectively. The control mycelial growth diameter was 33.6 ± 0.57 – 37.6 ± 0.28 mm. Kumar and Tyagi (2013) reported antifungal activity against all the six fungal species *A. niger*, *A. alternate*, *C. gloeosporioides*, *F. oxysporum*, *G.lucidum* and *R. solani* using different concentration of *Bergenia* stracheyi using poison food technique method. Bhattacharaya *et al.,* 2010 reported that the leaves of *Coccinia grandis* (L.) Voigt showed antifungal activity against *C. albicans* (750µ/ml) and *A. niger* (1000µg/ml) in ethyl acetate extracts. AS Apu *et al.,* (2010) reported that the leaves of *Dillenia indica* showed antifungal activity against some fungi such as *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae.* Chaithra *et al.,* (2013) finding showed that both leaf and rhizome extracts of *D. hidpida* showed most effective nearly double activity than the reference drug *Nystatin* with 10.2 to 12.1 mm on both the tested fungal strains. *A. niger* 19-29.5 mm and *C. albicans* 20-31.3 mm of zone of inhibition. And it is also observed *C. albicans* is most susceptible than *A. niger* with methanol and alcohol extracts followed by hot water and cold water extracts.

CONCLUSION

The present study confirms that leaf extracts from *D. gangeticum* (L.) DC., *C. peniculatus* Willd., *D. indica* Linn., *S. urens* Roxb. and tuber extracts of *D. hispida* Dennst. showed significant antifungal activity against *Aspergillus niger* ($P \leq 0.05$). Extensive research in the area of isolation and characterization of the bioactive compounds of these plants is required so that better, safer and cost effective drugs for treating fungal infections can be developed. Also recommended that further investigation is required for clinical trials using animal models for the betterment of humanity. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Thus, leading to opening up a new path in use of all five medicinal plants as a source of new antifungal agents and as a natural source for drug designing.

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Table 1. Effect of different concentrations of crude leaf extracts from *D. gangeticum* (L.) DC., *C. peniculatus* Willd., *D. indica* Linn., *S. urens* Roxb. and tuber extracts of *D. hispida* Dennst. on growth inhibition % against *Aspergillus niger*. Each value represents the mean of three measurements ± std. error from three petriplates having a similar amount of crude plant extracts. Significant difference at the 5% level between values obtained under control and the different concentration of extracts treatments (P≤0.05), according to DMRT.

Plant Species	Conc. (in µl)	Growth inhibition % (Mean (mm) ± Std. error)				
		Aqueous (DW)	Acetone	Chloroform	Petroleum ether	Ampho- B
DG	100µl	63.11 ± 4.23^{ef}	83.55 ± 1.17^{abcd}	60 ± 1.53^{f}	16.89 ± 11.58^{h}	64.88 ± 1.17^{ef}
	250µl	67.11 ± 2.70^{def}	85.77 ± 0.44^{abc}	69.33 ± 2.03^{cdef}	16.89 ± 11.58^{h}	79.55 ± 0.44^{abcde}
	500µl	70.22 ± 1.78^{bcdef}	87.11 ± 0.44^{ab}	68.44 ± 1.17^{cdef}	20 ± 12.02^{h}	85.77 ± 0.88^{abc}
	1000µl	73.33 ± 0^{bcdef}	91.55 ± 0.44^{a}	76.89 ± 3.55^{abcdef}	39.55 ± 10.04^{g}	87.55 ± 1.77^{ab}
CP	100µl	25.77 ± 20.45^{e}	29.33 ± 25.33^{de}	80.89 ± 7.11^{ab}	43.11 ± 8.48^{abcde}	64.88 ± 1.17^{abcde}

	250µl	33.33 ± 17.63^{bcde}	30.22 ± 26.22^{cde}	82.22 ± 5.78^{ab}	57.33 ± 6.16^{abcde}	79.55 ± 0.44^{abc}
	500µl	39.11 ± 17.56^{abcde}	31.11 ± 27.11^{cde}	84.89 ± 5.24^{a}	78.66 ± 4.80^{abcd}	85.77 ± 0.88^{a}
	1000µl	48.88 ± 13.51^{abcde}	31.11 ± 27.11^{cde}	86.66 ± 4.07^{a}	81.77 ± 3.10^{ab}	87.55 ± 1.77^{a}
DI	**100µl**	32.88 ± 13.77^{def}	4.89 ± 2.35^{f}	82.66 ± 4^{ab}	26.22 ± 19.57^{ef}	64.88 ± 1.17^{abcd}
	250µl	39.11 ± 16.26^{cdef}	5.33 ± 2.03^{f}	83.55 ± 3.79^{ab}	27.55 ± 19.57^{ef}	79.55 ± 0.44^{ab}
	500µl	47.55 ± 19.49^{bcde}	7.55 ± 1.176^{f}	85.77 ± 4.88^{a}	32.89 ± 18.48^{def}	85.77 ± 0.88^{a}
	1000µl	64 ± 8.88^{abcd}	70.66 ± 1.53^{abc}	85.77 ± 4.88^{a}	37.78 ± 17.77^{cdef}	87.55 ± 1.77^{a}
SU	**100µl**	4.89 ± 0.88^{h}	76.89 ± 5.40^{abc}	24.44 ± 14.57^{defgh}	38.22 ± 16.44^{cdefgh}	64.88 ± 1.17^{abcd}
	250µl	6.22 ± 0.44^{gh}	79.55 ± 3.87^{ab}	27.55 ± 16.26^{defgh}	$46.22 \pm 20.44^{bcdefgh}$	79.55 ± 0.44^{ab}
	500µl	9.77 ± 1.93^{fgh}	84.89 ± 3.10^{ab}	38.22 ± 19.01^{cdefgh}	46.66 ± 20^{abcdefg}	85.77 ± 0.88^{ab}
	1000µl	20.89 ± 6.94^{efgh}	86.22 ± 2.70^{ab}	48.44 ± 20.54^{abcdef}	51.11 ± 22.22^{abcde}	87.55 ± 1.77^{a}
DH	**100µl**	89.77 ± 0.44^{a}	32.44 ± 14.73^{ef}	12.44 ± 3.95^{f}	33.77 ± 11.58^{ef}	64.88 ± 1.17^{abcd}
	250µl	90.66 ± 0^{a}	39.55 ± 16.88^{de}	33.33 ± 10.18^{ef}	37.77 ± 12.37^{ef}	79.55 ± 0.44^{ab}
	500µl	91.55 ± 0.44^{a}	44 ± 18.92^{cde}	67.55 ± 2.70^{abc}	53.33 ± 3.85^{bcde}	85.77 ± 0.88^{a}
	1000µl	92 ± 0^{a}	70.66 ± 4.80^{ab}	82.22 ± 0.89^{a}	55.55 ± 4.44^{bcde}	87.55 ± 1.77^{a}

DG: *Desmodium gangeticum* (L.) DC.; **CP:** *Celastrus peniculatus* Willd.; **DI:** *Dillenia indica* Linn.; **SU:** *Sterculia urens* Roxb.; **DH:** *Dioscore hispida* Dennst.

D. gangeticum (Fabaceae)	***C. peniculatus*** (Celastraceae)	***D. indica*** (Dilleniaceae)	***S. urens*** (Sterculiaceae)	***D. hispida*** (Dioscoreaceae)

Photoplate 1: Selected Medicinal plants for the present study.

D. gangeticum (Acetone) leaf extracts	***C. peniculatus*** (Chloroform) leaf extracts	***D. indica*** (Chloroform) leaf extracts	***S. urens*** (Acetone) leaf extracts	***D. hispida*** (Aqueous) tuber extracts
				
				

Photoplate 2: Growth inhibition percent (%) of different solvents aqueous, acetone, chloroform, petroleum ether extracts at 1000µl against *Aspergillus niger*.

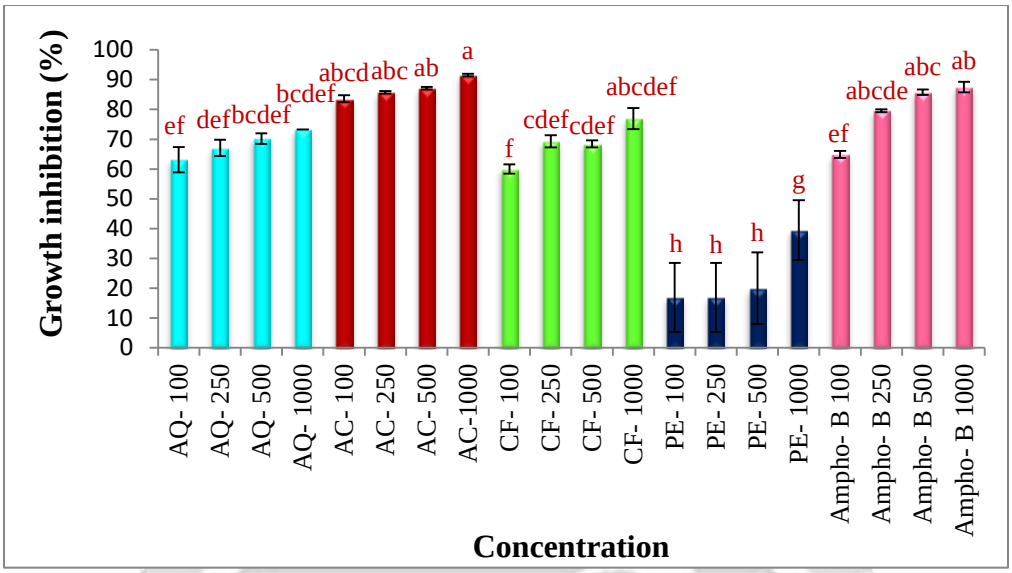

Fig 1. Effect of *D. gangeticum* crude leaf extracts on *Aspergillus niger*: ANOVA summary (F= 19.79; df= 19,40; p≤0.05). Means having similar alphabets, are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

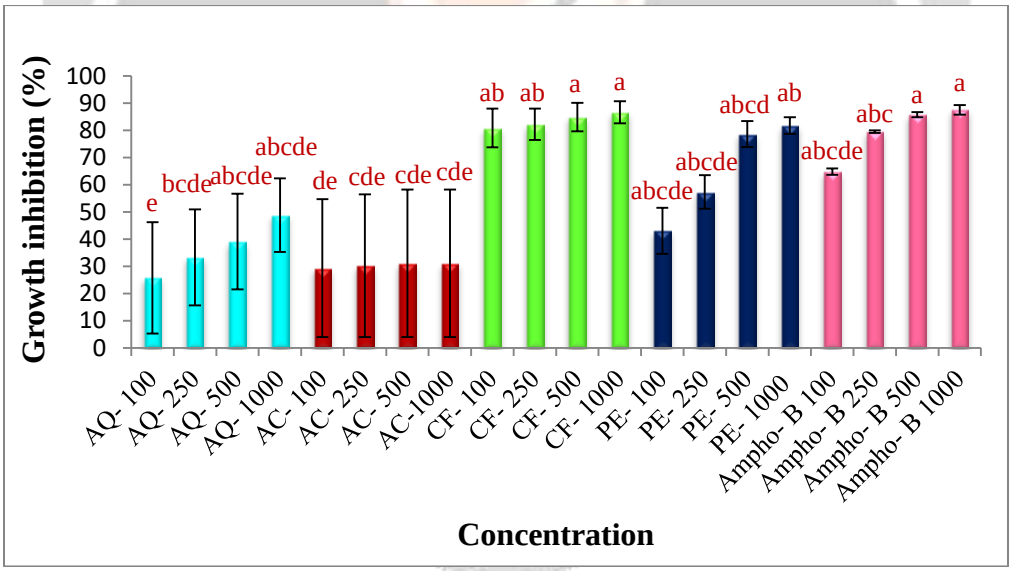

Fig 2. Effect of *C. peniculatus* crude leaf extracts on *Aspergillus niger*: ANOVA summary (F= 2.727; df= 19,40; p≤0.05). Means having similar alphabets, are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

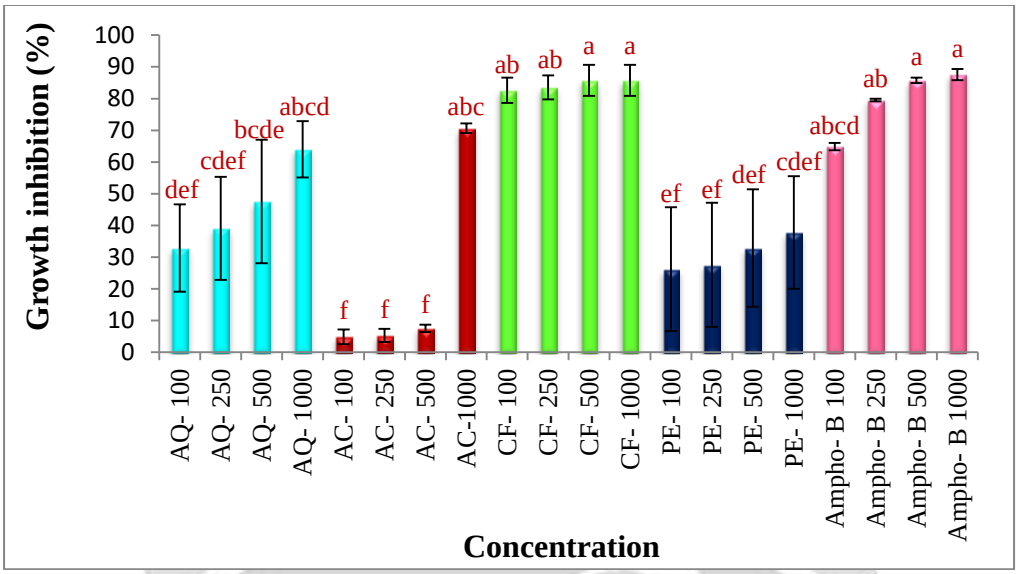

Fig 3. Effect of *D. indica* crude leaf extracts on *Aspergillus niger*: ANOVA summary (F= 7.26; df= 19,40; p≤0.05). Means having similar alphabets, are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

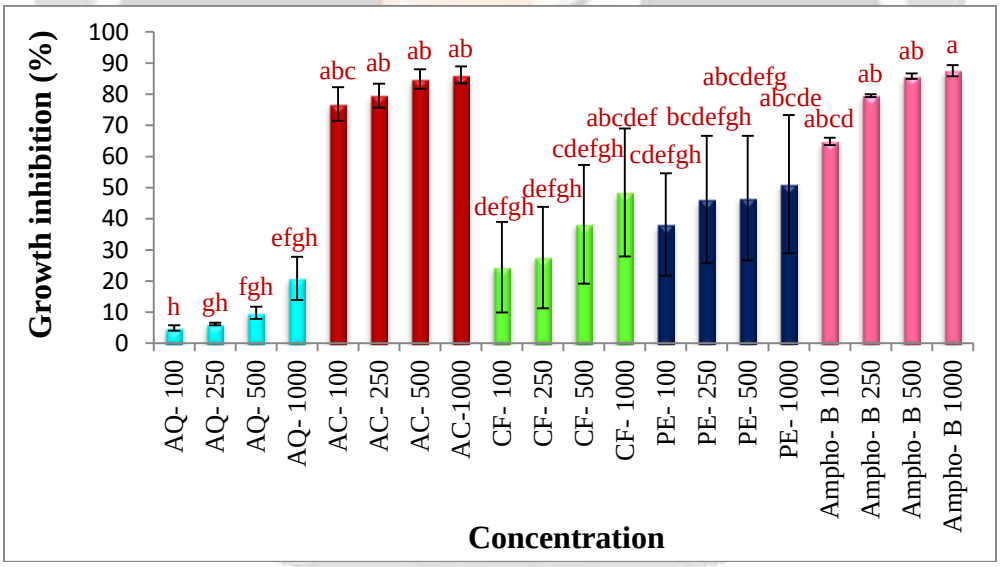

Fig 4. Effect of *S. urens* crude leaf extracts on *Aspergillus niger*: ANOVA summary (F= 5.61; df= 19,40; p≤0.05) Means having similar alphabets, are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).

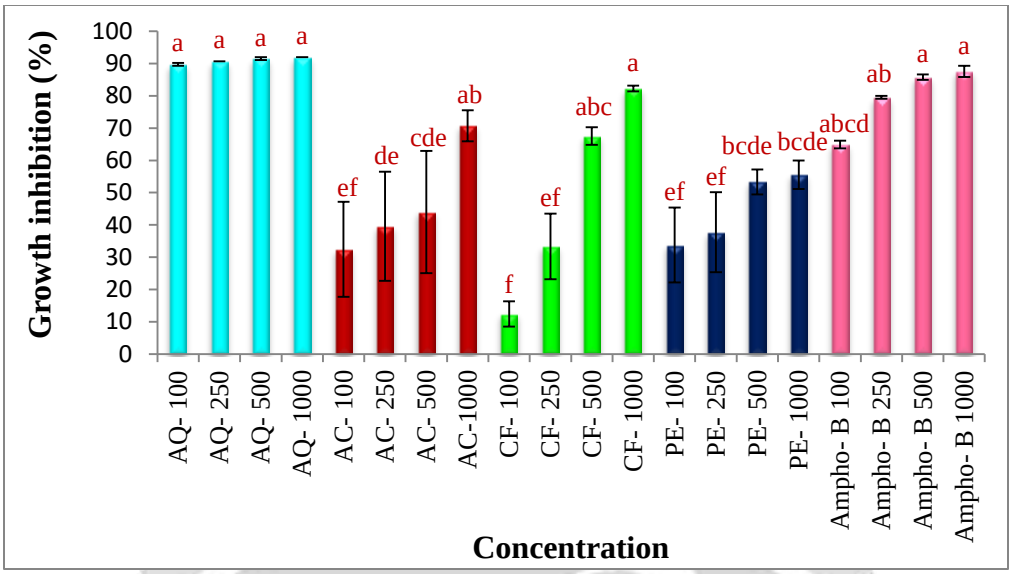

Fig 5. Effect of *D. hispida* tuber crude extracts on *Aspergillus niger*: ANOVA summary (F= 9.354; df= 19,40; p≤0.05). Means having similar alphabets, are not statically significant from each other at p≤0.05 (Based on Duncan's test, DMRT).