In-vitro evaluation of Fungi-toxic activity of *Ziziphus mauritiana* Lam.

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Abstract: Commonly known as Indian Ber, *Ziziphus*plants (Rhamnaceae family) are reported to possess bioactive compounds, recognized for traditional use and medicinal importance. Present study was aimed to evaluate the fungi-toxic properties of *Ziziphus mauritiana* Lam. have very nutritious fruits and are usually eaten fresh. Extracts of Barks, Leaves and Fruits were evaluated *in vitro* against four pathogenic fungi *viz. Aspergillus fumigates, Candida albicans, Alternaria solani* and *Rhizoctonia solani*. Bark, leaves and fruits extracts were prepared in three solvents *ie.* ethanol, methanol and hot water. Extracts of fruits showed complete (100%) mycellial inhibition of *Candida albicans* at 5% concentration which was comparable with respective fungicide at 100 ppm. Irrespective of plant's parts extracts prepared in ethanol was most effective in arresting the mycelial growth of the fungal pathogens whereas, superior fungi-toxic activity was observed in fruits extracts, followed by leaves and bark extracts.

IndexTerms **- Medicinal plants, Fungi toxic properties,*Ziziphusmauritiana,* Pathogens.**

I. INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plant antimicrobials offer potentially new classes of agents as they can substitute for synthetic antibiotics and drugs (Kothari *et* al. 2010). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to plant extracts and biologically active compounds isolated from plant species in herbal medicines (Essawi & Srour, 2000). Many of the plant products have important therapeutic agents, which are represented by the various phytochemicals like alkaloids, glucosides, flavanoids, mucilage, enzymes etc. Even now, drugs from higher plants continue to occupy an important niche in modern medicine.

Effective management of plant diseases are generally achieved by the use of synthetic pesticides (Bhanti &Taneja, 2005;Masoko&Eloff, 2006; Wang et al., 2008;Malinowska*et al*., 2015). However, most of the synthetic fungicides are non-biodegradable and pose problems to non-target organisms and their environment including humans. Though a large number of synthetic pesticides including fungicides have been banned in the western world yet in developing countries such as India, these are still being used despite their detrimental effects (Wodageneh and Wulp, 1997). On the other hand many plant pathogenic micro-organisms have developed resistance against known chemical pesticides (Urech *et al.,* 1997; Williams and Heymann, 1998; Witte, 1998). For this reason attention has been diverted to an alternative, safe and cheap method for the management of fungal pathogens. Fungi toxic, fungi static and fungicidal activity of the medicinal plants on plant pathogens has been widely reported (Cho *et al*., 2006; Das *et al*., 2010a, 2010b; Shrivastava *et a*l., 2012; Thippeswamy *et al.,* 2013; Gupta *et al*., 2015).

Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. One such medicinal plant is *Ziziphus mauritiana* Lam., a member of the family Rhamnaceae commonly known as ber and is mostly found almost in all parts of the country. *Ziziphus mauritiana* Lam. is a low branched deciduous tree with spreading crown, dark greenish black bark having irregular crack and strong reddish hardwood with oblong and elliptic leaves. In traditional medicine, the leaves, fruits, bark & even roots are used to treat a variety of ailments including cold, flu and malnutrition related diseases in children, convulsions and indigestion (Mazumder *et al.,* 2004). The leaves are applied as poultices and are helpful in liver troubles, asthma, and fever and to treat sores (Michel, A. 2002) and the roots are used to cure and prevent skin diseases (Adzu *et al.,* 2001). All the parts of this plant are very effective against different types of diseases. Its leaves are useful in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea. The leaves of *Z. mauritiana* are also used in the treatment of liver diseases, asthma and fever. The fruit has been used as anodyne, sedative, tonic anticancer and potent wound healer. The fruit (Ndhala *et al.,* 2006) leaves (Dahiru and Obidoa, 2007) and seed (Bhatia and Mishra, 2009)extracts have been shown to exhibit antioxidant activity, whereas barkis reported to have cyto-toxicity against different cancer cell lines.

Considering the vast potentiality of *Ziziphus mauritiana*as sources for antimicrobial drugs and / or fungicides this study was undertaken to evaluate the fungi-toxicity of *Z. mauritiana* leaves and fruits against human and plant pathogenic fungi.

II. MATERIALS AND METHODS

2.1Isolation of pathogens

Alternaria solani and *Rhizoctonia solani* were isolated from naturally infected plant parts. Infected plant materials were washed-cut into 5 mm segments and surface disinfected in 0.5% Sodium Hypo-chloride solution for 5 min followed by washing in three changes of sterile distilled water. The segments were dried in between sheets of sterile filter paper and plated on fresh

sterilized Potato Dextrose Agar (P.D.A.) medium impregnated with Streptocycline (100 ppm), and incubated for 7 days at 26 ±1°C. Pure culture was obtained by sub-culturing three times and maintained on PDA slants in the refrigerator until required. *Aspergillus fumigates* and *Candida albicans* were borrowed from authorized clinical pathology centre in pure culture form and through three times sub-culturing maintained on Sabouraud's dextrose agar slants in the refrigerator until required.

2.2 Collection of plant materials and preparation of the plant extract

Fresh leaves and fruits were collected from the college campus and local fields. Fresh plant materials were washed and surface sterilized (Mercuric Chloride, 0.01%) and chopped finely in a blender before the extraction (Kurucheve*et al*., 1997). The plant materials were plunged in required quantity of solvent (1:1 w/v) in a glass beaker, heated over a hot plate at 90-100°C (45-50min) for hot water and kept overnight at room temperature for organic solvent (ethanol, methanol and hot water) extraction respectively. The pulp of the plant tissue along with the extraction solvent were then squeezed and filtered through 3 layers of muslin cloths followed by low speed centrifugation (5000 rpm for 5 min) to get the clear supernatant (Priya and Ganjewala, 2007). The residual solvents were evaporated at room temperature. Plant extracts thus obtained was crude standard stock solution (Tiwari *et al*., 2005).

2.3 Fungi toxic activity of crude plant extract on fungal growth*in vitro*

Fungi-toxic efficacy of plant extracts were evaluated in vitro, adopting poisoned food technique (Grover and Moore, 1962). Crude plant extract stock solution were used at three concentrations (25%, 50%, 75%) prepared by in mixing aseptically 5ml, 10ml and 20ml of stock solution in 100ml of semisolid sterilized potato dextrose agar medium (Tiwari *et al*., 2005) at a temperature of 40°-45°C. Potato dextrose agar medium alone, with different solvents and with fungicide Carbendazim (Bavistin) at 100ppm concentration served as positive, solvent and negative control respectively. Plates with gelled medium were inoculated in the center with 7mm diameter mycelial disk of 3 days old fungal culture. All the plates were then incubated at 26 ±1°C and mycelial growth was recorded after 96 hrs of incubation. Colony diameter is recorded twice perpendicularly. Percent inhibition of mycelial growth was calculated using the formula given below (Verma and Kharwar, 2006).

% inhibition = 100 X Mycelial growth (control) - Mycelial growth (treatment) / Mycelialgrowth (control)

2.4Experimental design and statistical analysis

All the experiments were arranged in completely randomized design with three replications. Data were analyzed statistically with the help of software and mean values were considered with ±SD. Average percent Mean mycelial inhibition of solvent extracts and the trial means of medicinal plants were calculated for average.

III.RESULT AND DISCUSSION

A significant variability was observed in antifungal property of plant parts within different solvent of extracts at varying concentrations against four fungal pathogens i.e. *Aspergillus fumigates, Candida albicans, Alternaria solani* and *Rhizoctonia*

TABLE: Fungi toxicity of leaf extract in different concentration (25%, 50% & 75%) of *Z. mauritiana* leaves and fruits in different solvent (Ethanol, Methanol and Hot water) as measured by percent mycelia inhibition (Mean ± SD) of *Aspergillus fumigates, Candida albicans, Alternaria solani* and *Rhizoctonia solani.*

Fungal species	Extracts	Percent Mycelial Inhibition (%)												Fungicide (Bavistin)
		Bark				Leaves				Fruits				
		25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%	100 ppm
Aspergillus fumigatus	*Ethanol*	64.8 ±0.31	71.6 ±0.02	82.5 ±0.14	87.6 ±0.23	72.4 ±0.53	81.5 ±0.36	89.3 ±0.06	92.6 ±0.08	76.7 ±0.67	84.5 ±0.36	91.7 ±0.06	96.2 ±0.32	100.00 ±0.00
	Methanol	52.6 ±0.34	68.4 ±0.36	73.6 ±0.24	81.6 ±0.25	52.9 ±0.42	69.4 ±0.23	75.8 ±0.18	84.2 ±0.09	63.2 ±0.26	75.4 ±0.14	84.3 ±0.18	90.4 ±0.24	100.00 ±0.00
	Hot water	38.4 ±0.34	46.8 ±0.36	53.6 ±0.24	65.7 ±0.25	43.4 ±0.37	56.3 ±0.33	67.8 ±0.32	73.6 ±0.23	51.3 ±0.14	68.5 ±0.09	77.3 ±0.26	85.3 ±0.19	100.00 ±0.00
	Trail Mean	51.9 ±2.63	62.2 ±3.42	69.9 ±3.84	78.3 ±2.17	56.2 ±3.21	69.0 ±2.48	77.6 ±1.92	83.4 ±2.14	63.7 ±3.29.	76.1 ±2.53	84.4 ±1.86	90.6 ±1.78	100.00 ±0.00
Candida albicans	*Ethanol*	68.23 ±0.21	79.4 ±0.32	87.8 ±0.41	92.6 ±0.08	66.7 ±0.24	81.3 ±0.21	86.7 ±0.08	93.7 ±0.17	68.3 ±0.26	81.3 ±0.31	96.7 ±0.27	100.0 ±0.14	100.00 ±0.00
	Methanol	55.6 ±0.16	68.5 ±0.23	74.8 ±0.32	83.7 ±0.12	56.8 ±0.24	72.8 ±0.18	81.6 ±0.31	89.6 ±0.14	58.7 ±0.15	70.4 ±0.24	84.3 ±0.33	93.7 ±0.17	100.00 ±0.00
	Methanol	41.7 ±0.23	53.6 ±0.19	65.8 ±0.22	72.4 ±0.17	46.5 ±0.22	58.3 ±0.26	76.4 ±0.21	81.6 ±0.17	48.3 ±0.32	66.4 ±0.16	77.3 ±0.15	87.2 ±0.31	100.00 ±0.00
	Trail Mean	55.1 ±2.18	67.1 ±2.08	76.1 ±1.94	82.9 ±1.78	56.6 ±2.04	70.8 ±2.35	81.5 ±1.43	88.3 ±1.62	58.4 ±1.46	72.7 ±1.83	86.1 ±1.64	93.6 ±0.94	100.00 ±0.00
Alternaria solani	*Ethanol*	54.7 ±0.31	69.4 ±0.02	73.7 ±0.28	84.5 ±0.08	55.7 ±0.67	68.8 ±0.36	77.4 ±0.06	90.2 ±0.24	49.8 ±0.67	71.6 ±0.36	85.8 ±0.06	92.5 ±0.16	100.00 ±0.00
	Methanol	48.4 ±0.26	57.5 ±0.18	65.4 ±0.23	74.3 ±0.13	50.4 ±0.24	62.3 ±0.18	73.6 ±0.14	83.5 ±0.17	54.6 ±0.27	68.6 ±0.18	75.8 ±0.16	86.2 ±0.24	100.00 ±0.00
	Hot water	29.6 ±0.34	40.4 ±0.31	51.7 ±0.26	60.6 ±0.33	32.7 ±0.34	44.3 ±0.18	55.2 ±0.24	64.8 ±0.26	50.5 ±0.31	63.4 ±0.22	72.6 ±0.34	80.5 ±0.24	100.00 ±0.00
	Trail Mean	44.2 ±2.24	55.7 ±1.86	63.6 ±2.18	73.1 ±2.24	46.2 ±2.73	58.4 ±3.48	68.7 ±2.18	79.5 ±1.86	51.6 ±2.08	67.8 ±1.78	78.0 ±2.24	86.4 ±1.67	100.00 ±0.00
Rhizoctonia solani	*Ethanol*	57.3 ±0.21	65.4 ±0.08	74.8 ±0.16	87.2 ±0.27	60.3 ±0.52	73.7 ±0.41	81.3 ±0.02	90.2 ±0.05	64.3 ±0.52	81.3 ±0.41	89.3 ±0.07	96.7 ±0.22	100.00 ±0.00
	Methanol	50.6 ±0.32	58.4 ±0.17	65.6 ±0.27	74.8 ±0.21	52.7 ±0.41	65.5 ±0.35	72.6 ±0.16	84.3 ±0.17	56.7 ±0.32	69.4 ±0.36	78.6 ±0.17	85.4 ±0.24	100.00 ±0.00
	Hot water	34.7 ±0.16	43.8 ±0.12	52.4 ±0.27	60.5 ±0.16	39.4 ±0.36	58.3 ±0.32	68.4 ±0.24	77.4 ±0.32	44.3 ±0.16	55.8 ±0.24	66.5 ±0.27	75.6 ±0.16	100.00 ±0.00
	Trail Mean	47.5 ±2.32	55.8 ±2.17	64.2 ±1.84	74.1 ±1.36	50.8 ±2.27	65.8 ±2.05	74.1 ±1.84	83.9 ±1.42	55.1 ±2.14	68.8 ±1.74	78.1 ±1.83	85.9 ±14	100.00 ±0.00

solani, as the observed variations in percent mycelia growth inhibition have been computed in TABLE -1. The concentration of different solvent extracts increased; in case of different parts of the plant, the efficacy against all four pathogens were also increased significantly (TABLE-1 and Fig. 1 to 4). Complete inhibition (100%) of mycelial growth was recorded in ethanol extracts of fruits against *Candida albicans* and also observed in respective fungicide (Bavestin at 100 ppm) treatment, while more 90 % inhibition of mycelia growth in *Candida albicans* were observed due to ethanol extracts of bark, leaves and fruits of *Ziziphus mauritiana.*

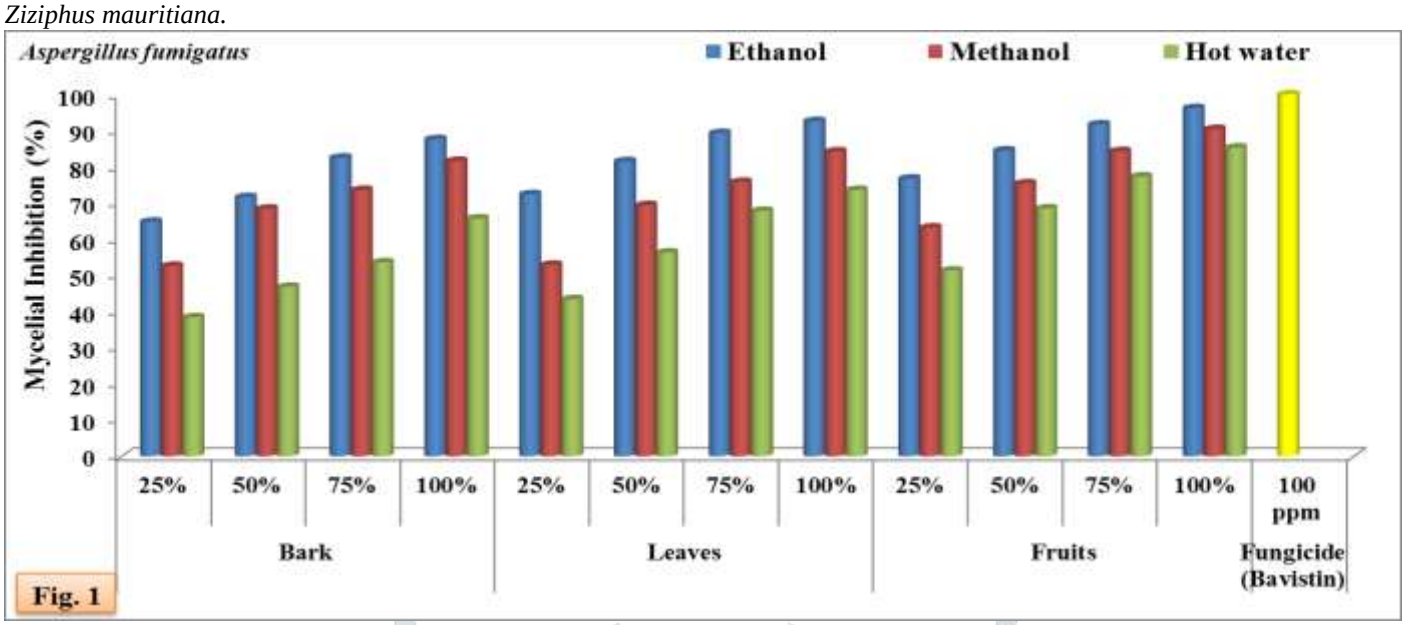

Fig. 1

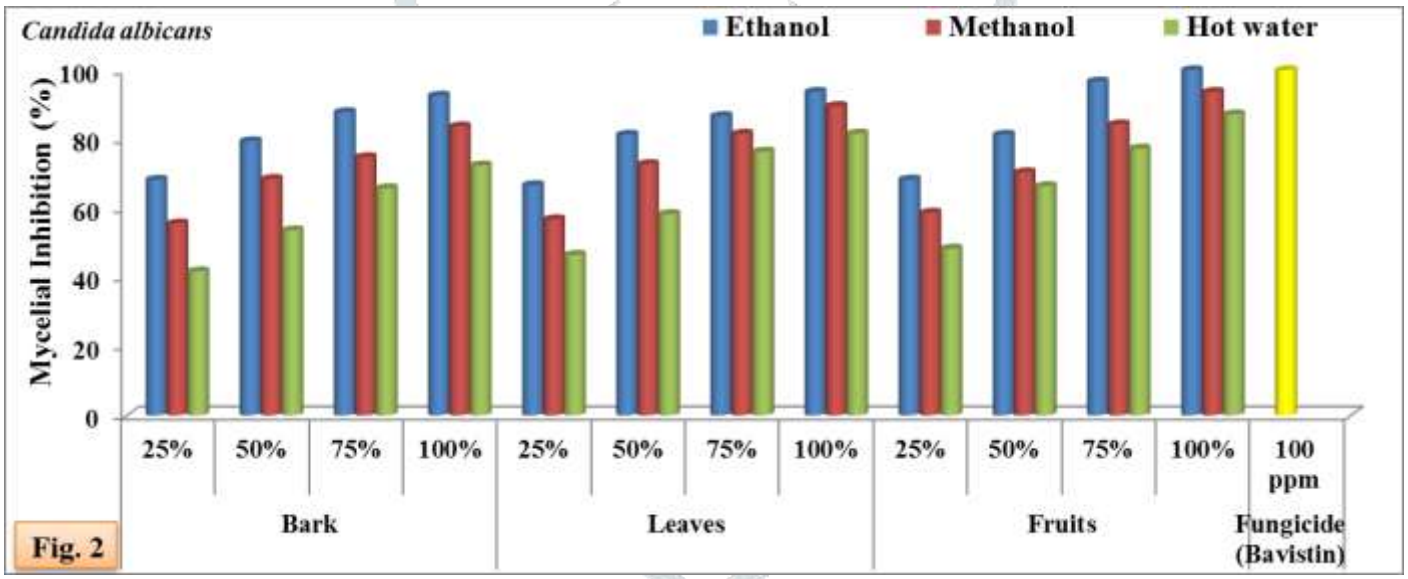

Fig. 2

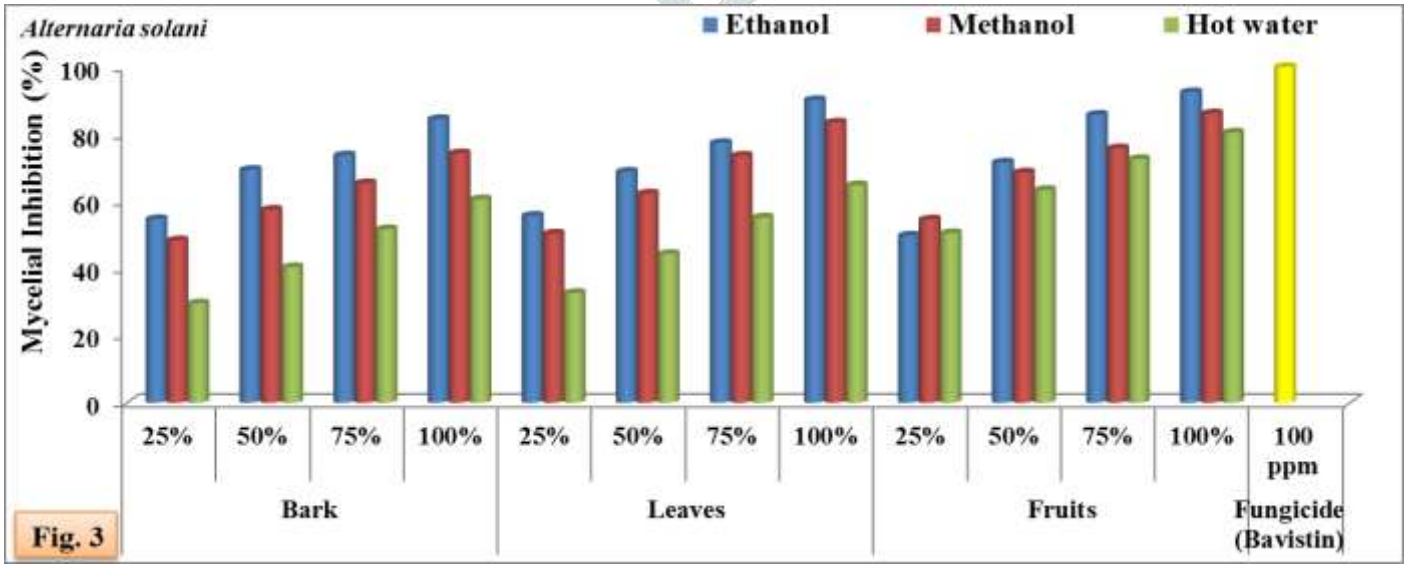

Fig. 3

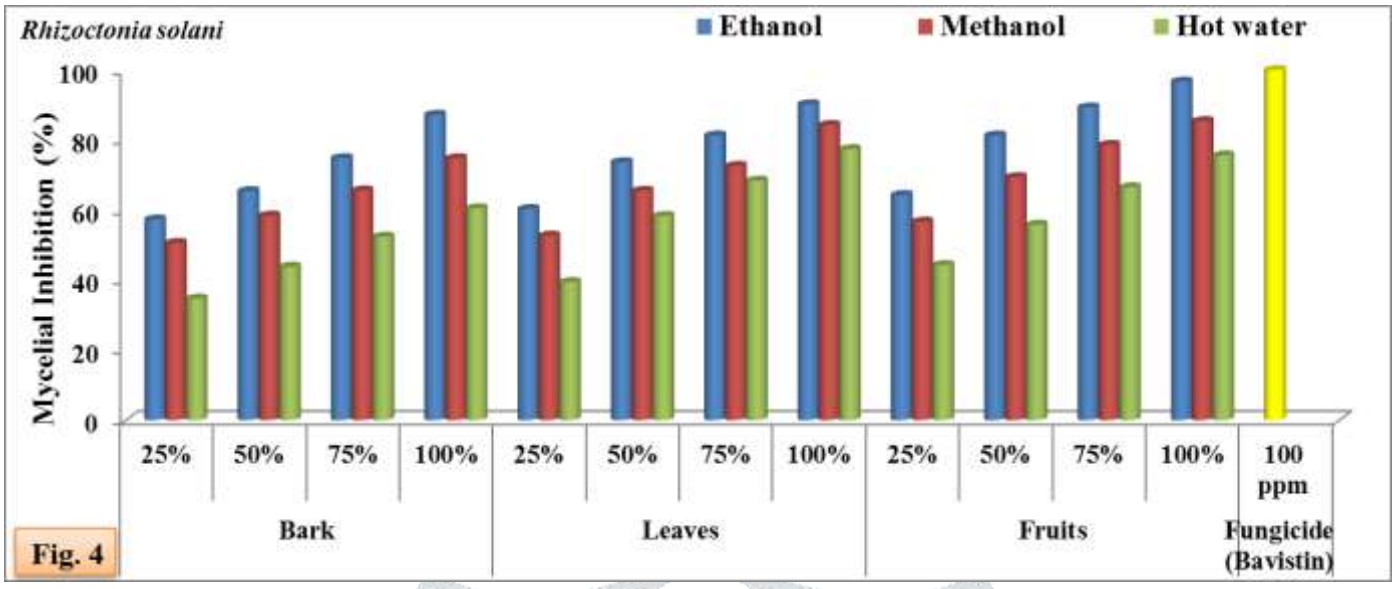

Fig. 4

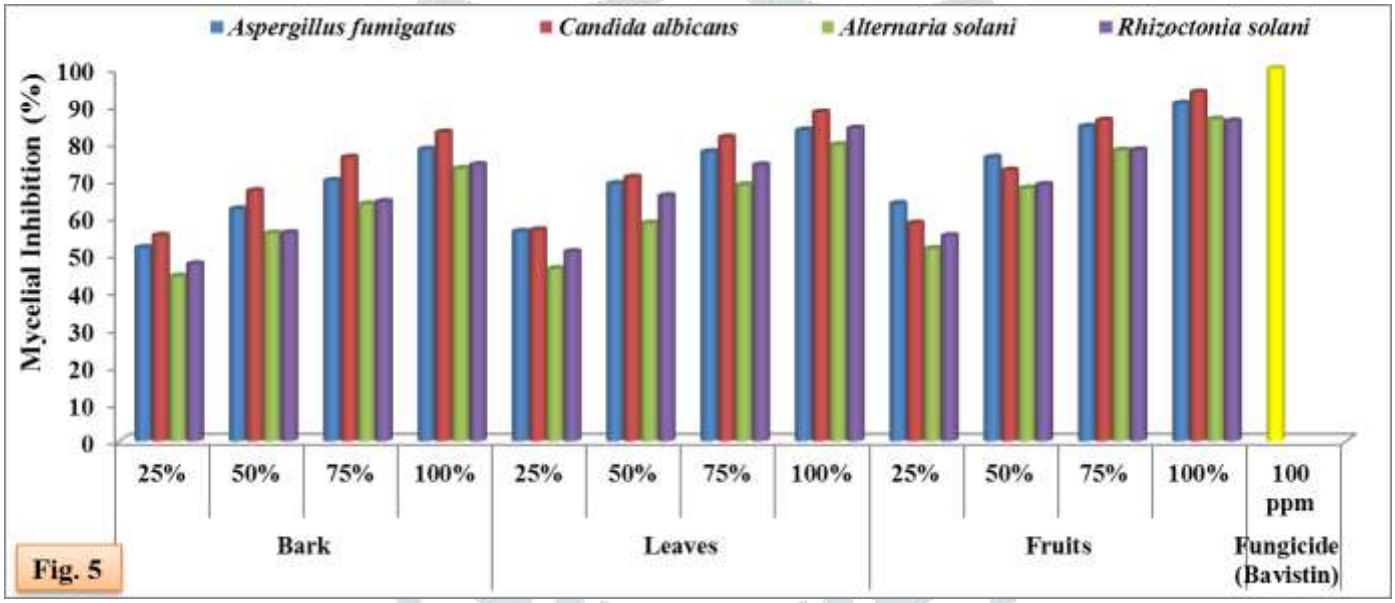

Fig. 5

Greater efficacy of *Ziziphus mauritiana*in terms of mycelial inhibition at different concentration was recorded for fruits extract prepared in ethanol i.e. 100% against *Candida albicans*, 96.7 % against *Rizoctonia solani*, 96.2% against *Aspergillus fumigates* and 92.5% against *Alternaria solani*, followed by methanol and hot water. Anti-fungal efficacy of fruits extracts were observed greater in different concentrations against all four pathogens followed by leaves and bark,whereas against *Candida albicans*, it was observed more than *Rizoctonia solani* followed by *Aspergillus fumigatus* and *Alternaria solani* (Fig. 5) respectively. Fungi-toxic potentiality of bark, leaves and fruits of *Ziziphus mauritiana* were compared with fungicides (Bavestin, 100ppm) and observed significant result against pathogenic fungi, especially ethanol extracts of fruits and leaves (Fig. 1 to 4) that is similar findings of previous investigations (Bhatia and Mishra, 2009; Gupta *et al*., 2015).

IV.CONCLUSION

Findings of the present investigation reveals that the fruits, leaves and bark extracts of *Ziziphus mauritiana* wild plant has potential to yield potent drugs /fungicide compound targeted to pathogenic fungi. This study concluded that Fruits, Leaves extract of *Z. mauritiana* possess antifungal property may be due to aromatic compounds as the secondary metabolites, especially phenolic content although somewhat less then synthetic standard drug and /or bio-fungicides so formulations may be prepared with this extract as alternative to synthetic drugs / fungicides that may be sustainable efforts in the field of pharmacology to control the fungal diseases.

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