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Synergistic Effect of *Boerhaavia diffusa* and *Asparagus racemosus* against *Aspergillus fumigatus, Aspergillus niger, Fusarium moniliforme and Aspergillus terreus*

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ABSTRACT

The following study was carried out at D.E.I, Dayalbagh to study the synergistic effect of medicinal plants Boerhaavia diffusa and Asparagus racemosus against Aspergillus fumigatus, A. niger, A.terreus and Fusarium moniliforme. These plants were selected to evaluate the possibility of new fungicides. But there were results where these plants couldn't be seen effective against plant pathogenic fungi. These plants were also tested in synergism to see the possibility of finding such a combination which can prove effective against fungi. Therefore, in the present study individual and synergistic effects of plant extract were determined against different plant pathogenic fungi. According to the observation of the present work combined stem extract of both the plant shows positive synergism by exhibiting highest inhibitory effect in comparison to the individual extracts. Combination of methanolic stem extract of A. racemosus and B. diffusa showed positive synergism against Aspergillus fumigatus.

Keywords: Synergism, Antifungal activity, inhibitory effect etc

INTRODUCTION

Medicinal plants play a major role in the traditional medicine system by using herbal remedies for several ailments including infection, fever, malaria, inflammation etc. The plant material continues to be a part of mankind's primary health care as therapeutic remedies. Plants contain chemical components that, in addition to providing protection from diverse antagonists, act to potentiate each other's activities, and thereby economize resource allocation to defensive chemicals (Richards *et al.,* 2016). Fungi are microbes that cause a wide range of illnesses, from minor skin conditions to life-threatening diseases. Fungal infections can become fatal to people with immune compromised conditions particularly HIV/AIDS, cancer patients (M. Jankowska *et al.*, 2001). Use of herbal drugs for the control of opportunistic infections is considered as a promising solution and an interesting alternative to synthetic fungicides. Traditionally, the prepared therapy involves either a single herb, or combination of herb(s) and conventional drug(s). When herbs are used in combination, the effects can be complicated as various interactions can occur among the individual components. The most desired interactions in the body are those which can result in additional therapeutic benefits; however, they can also reduce activity or cause toxicity (CT Che *et al.*, 2013).

Synergistic effects occur when two compounds increase one another's potency, resulting in mixtures that have stronger effects than predicted based on activities of their components in isolation. Synergistic effects are especially useful in clinical situations. By reducing the dose required to achieve a medical effect, selectively synergistic drug combinations can both reduce costs and lower the risk of patient toxicity (Greco *et al.,* 1995). There are few different classes of effective antifungal drugs available for treatment of fungal diseases of plants, animals and humans. Thus, it is important to develop new sources of antifungal compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases as well as resistance to currently used drugs. The investigations on new antifungal substances should be continued and all possible strategies and techniques need to be explored further. Plants, which have evolved to produce defensive mixtures under conditions of limited resources and diverse antagonists, are an intuitive place to look for synergistic chemical combination (Richards *et al.,* 2016).Generally speaking, synergy can occur when one compound

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increases the bioavailability (Smith, Roddick & Jones , 2001), inhibits detoxification (Berenbaum & Neal, 1985). There are limited reports on the antifungal effects of plant parts or plant extracts of *Asparagus racemosus* and *Boerhaavia diffusa*. Present study was therefore undertaken to evaluate ability of these plant extracts against selected fungal strains namely *Aspergillus fumigatus*, *Fusarium moniliforme*, *Aspergillus niger, Aspergillus terreus* individually and in synergism. Plant origin herbal fungicides are considered as safe alternatives to synthetic drugs. Antifungal activity means destroying fungi or inhibiting their growth. Fungal species since time immemorial have been affecting plants and crops which cause loss at larger scale, not only this but also causes various diseases and is a big problem for mankind. Recent researches have focused on plant products that act as an alternative to existing drugs against plant pathogenic fungi.

MATERIALS AND METHODS

Pathogenic microbes were inoculated in petri dishes containing sabround dextrose agar directly and were incubated at suitable temperature 27^{o}C. Colonies were transferred to other petri dishes for purification. Revival of pathogens: Pathogenic fungi was revived in nutrient broth and stored in BOD incubator for 2 days at 27^{0} C and maintained in slants at 4^{0}C. The fully mature *Asparagus racemosus* and *Boerhaavia diffusa* leaves stem and roots were collected from herbal garden (D.E.I Dayalbagh Agra). Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant materials using filter paper followed by drying in shade. After drying, powder of the dried plant parts was grinded by using mortar pestle and was stored in an airtight container for further use.

Extract preparation Fresh plant materials of *Asparagus racemosus* and *Boerhaavia diffusa* were grinded using mortar pestle. For individual plant extract 2 gm of fresh plant parts (root, stem and leaves) were grinded in 20 ml distilled water separately. It was then filtered using muslin cloth, stored in falcon tubes and used within 24 hrs.

Ethanolic extract Fresh plant materials were grinded using mortar pestle. For individual plant extract 2gm of fresh plant parts (root, stem and leaves) were grinded in 20 ml ethanol. It was then filtered using muslin cloth, stored in falcon tubes and used within 24 hrs.

Methanolic extract Methanolic extract was used only for assessment of synergistic activity. For this, previously dried powder of leaf, root and stem of *Boerhaavia diffusa* and *Asparagus racemosus* were used. 2gm of mixed powder was added in 20 ml of methanol.

Assessment of synergistic effect of plant extract against different pathogenic fungal strains:

The antimicrobial effect of plant extract viz. *Asparagus racemosus* and *Boerhaavia diffusa* against the target pathogenic fungi was studied using –

Paper disc diffusion method (Pelezar *et al*, 1993; Okigbo *et al*, 2005):

Antimicrobial activity by disc diffusion method was carried out. Disc measuring 06mm in diameter were pinched from Whatman no.1 filter paper using a cork borer of fixed diameter. The discs, saturated with different extracts containing varying concentration were placed on SDA medium seeded with the test organism. Disc fed with corresponding solvent alone served as control. The plates were incubated at suitable temperature and observed for a zone of inhibition after 1-3 days.

RESULTS

Maintenance of fungal cultures

Fungal cultures used were *Aspergillus fumigatus, Fusarium moniliforme, Aspergillus niger, and Aspergillus terreus.* (Fig 1a-d) These cultures were obtained from Microbiology lab. Department of Botany, Dairy campus, D.E.I Agra. All the cultures were procured from the Indian Type Culture Collection (ITCC), IARI, New Delhi. Revival of pathogens: the fungi was revived in nutrient broth and stored in a BOD incubator for 2 days at 27^{0} C and maintained in slants at 4^{0}C.

2. Collection and Preparation of Aqueous extract of root, stem and leaves of *Boerhaavia diffusa* and *Asparagus racemosus.* Aqueous extract of different plant parts of the selected two plants were prepared as per method given previously.

Collection and preparation of fresh ethanolic

extract of root, stem and leaves of *Boerhaavia diffusa* and *Asparagus racemosus*. Ethanolic extract of the 2 selected plants were prepared using fresh parts as per methodology given.

For solvent extract Methanolic extracts of different plant parts of *Asparagus racemosus* and *Boerhaavia diffusa* root, stem and leaves were prepared by dissolving 2gm mixed powder in 20 ml methanol. Methanolic extracts were used only for evaluating synergistic effect of 2 selected plants against the selected fungal strain.

3. In-vitro assessment of antifungal activity of plants extracted against selected fungal strains individually and in synergism.

Antifungal activity of leaves of *Boerhaavia diffusa* and *Asparagus racemosus* individually and in combination was studied by paper disc diffusion method against *Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus and Fusarium moniliforme.* Stem combination of the plants exhibited highest zone of inhibition among combination of leaves and roots extract of *Boerhaavia diffusa* and *Asparagus racemosus* and Table 1-3.

Table 1: Antifungal activity of various parts of *Asparagus racemosus* against various fungal pathogens

Fungal strain	Plant part	Solvent	Results
A.fumigatus	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
F.moniliforme	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A. niger	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A. terreus	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A.fumigatus	Root	ethanolic	-
	Stem	ethanolic	2mm
	Leaves	ethanolic	-
	Control	Ethanolic	-
F.moniliforme	Root	ethanolic	-
	Stem	ethanolic	-
	Leaves	ethanolic	-
	Control	ethanolic	-
A.niger	Root	ethanolic	-
	Stem	ethanolic	-
	Leavers	ethanolic	-
	Control	ethanolic	-
A.terreus	Root	ethanolic	-
	Stem	ethanolic	-
	Leaves	ethanolic	-
	Control	ethanolic	-

Maximum antifungal activity of *Asparagus racemosus* was obtained against *Aspergillus fumigatus* in ethanolic extract of stem and no zone was observed in leaves and root. No zone of inhibition was observed in Aqueous and fresh ethanolic extracts of the *Aspergillus niger, Aspergillus terreus* and *Fusarium moniliforme.*

Table 2: Antifungal activity of various parts of *Boerhaavia diffusa* against various fungal pathogens

Fungal strain	Plant part	*Solvent*	*Results*
A.fumigatus	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
F.moniliforme	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A.niger	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A.terreus	Root	Aqueous	-
	Stem	Aqueous	-
	Leaves	Aqueous	-
	Control	Aqueous	-
A. fumigatus	Root	Ethanolic	-
	Stem	Ethanolic	5mm
	Leaves	Ethanolic	-
	Control	Ethanolic	-
F. moniliforme	Root	Ethanolic	-
	Stem	Ethanolic	-
	Leaves	Ethanolic	-
	Control	Ethanolic	-
A.niger	Root	Ethanolic	-
	Stem	Ethanolic	-
	Leaves	Ethanolic	-
	Control	Ethanolic	-
A.terreus	Root	Ethanolic	-
	Stem	Ethanolic	-
	Leaves	Ethanolic	-
	Control	Ethanolic	-

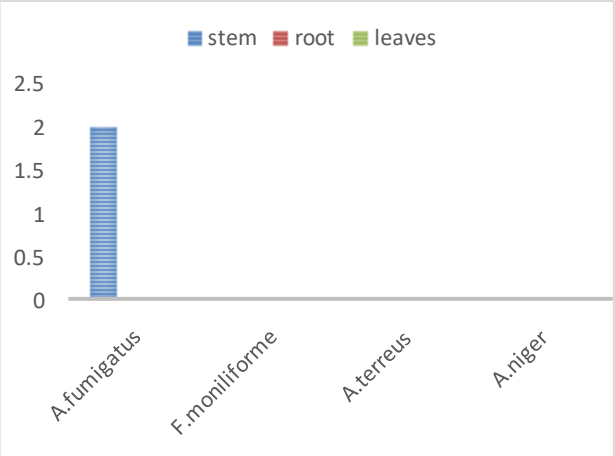
Maximum antifungal activity of *Boerhaavia diffusa* was obtained against *Aspergillus fumigatus* in ethanolic extract of stem and no zone was observed in leaves and root. No zone of inhibition was observed in aqueous extract and fresh ethanolic extract of the *Aspergillus niger, Aspergillus terreus* and *Fusarium moniliforme*.

Table 3: Antifungal activity of combined parts of *Asparagus racemosus* and *Boerhaavia diffusa* against *Aspergillus fumigates*

Plant part	Strain	Solvent	Inhibition zone
Root	*A.fumigatus*	Methanol	0 mm
Stem		Methanol	6 mm
Leaves		Methanol	0 mm

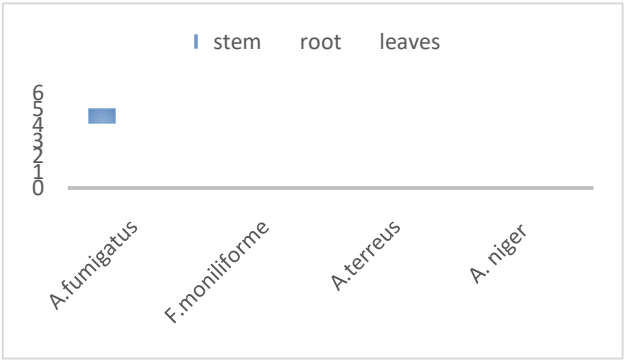
Since the individual plant parts of the selected plants exhibited positive results against *A.fumigatus* only. Therefore, to study the synergistic effect of the 2 plants viz. *Asparagus racemosus* and *Boerhaavia diffusa* was tested.Maximum antifungal activity of combined plant parts of *Asparagus racemosus* and *Boerhaavia diffusa* against *Aspergillus fumigatus* was observed in methanolic extract of stem whereas no zone of inhibition was observed in root and leaves.

Comparative study of both fungal strains against fresh ethanolic extracts of various parts of *Asparagus racemosus*.



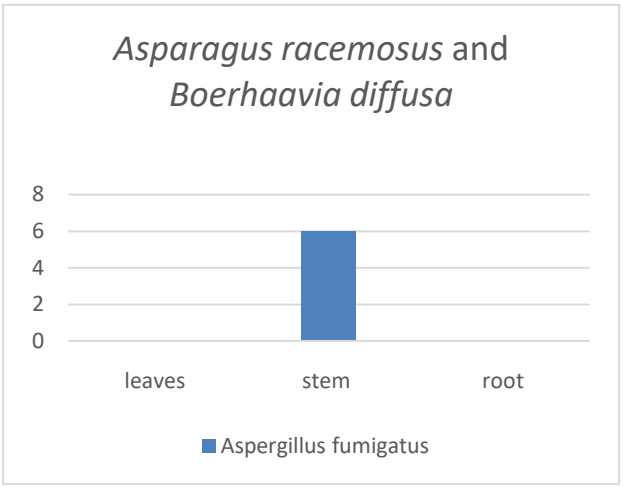
When ethanolic extracts of various plant parts of *Asparagus racemosus* were tested against all 4 selected fungi, it was observed that no inhibition zone was reported against *Fusarium moniliforme, Aspergillus terreus, Aspergillus niger* while inhibition zone (2mm) only in stem of *Asparagus racemosus* was seen in *Aspergillus fumigatus*

Comparative study of both fungal strains against fresh ethanolic extracts of various parts of *Boerhaavia diffusa*



Ethanolic extracts of various plant parts of *Boerhaavia diffusa* were tested against all the 4 selected fungi. No inhibition zone was reported against *Fusarium moniliforme, Aspergillus terreus and Aspergillus niger.* Stem extract of *Boerhaavia diffusa* only exhibited a 5mm inhibition zone against *Aspergillus fumigatus*

Comparative study of fungal strain *Aspergillus fumigatus* against methanolic extracts of various parts of combined plant *Asparagus racemosus* and *Boerhaavia diffusa.*



- Aqueous extracts of various plant parts of *Asparagus racemosus* were totally ineffective against *Fusarium moniliforme, Aspergillus terreus,* and *Aspergillus niger.* However aqueous extracts of stem of *Asparagus racemosus* showed moderate inhibition against *Aspergillus fumigates.*

- Aqueous extracts of various plant parts of *Boerhaavia diffusa* were totally ineffective against *Fusarium moniliforme, Aspergillus terreus* and *Aspergillus niger.* However aqueous extracts of the stem of *Boerhaavia diffusa* showed a high zone of inhibition against *Aspergillus fumigatus.*
- Methanolic extracts of combined stem extracts of *Boerhaavia diffusa* and *Asparagus racemosus* was highly effective against *Aspergillus fumigatus,* however combined extracts of roots and leaves of the same did not show any result against *Aspergillus fumigatus*

DISCUSSION AND CONCLUSION

Plants have proved to be significant natural resources for medicines; documentation of all their use in medicine originate from ancient times. Plants generally produce many secondary metabolites with antifungal and microbicidal activity. According to WHO (World Health Organization) plants would be the best source for obtaining a variety of drugs and a positive way to treat disease caused by multidrug resistant microorganisms (Bhattacharjee *et al.,* 2006).Ethnobotanical and ubiquitous plants provide a rich resource for natural drug research and development. Screening of medicinal plants for antifungal activities is important for finding potential new compounds that can be used as fungicides.

Present study includes two imp medicinal plants viz, *Asparagus racemosus* and *Boerhaavia diffusa,*. Medicinal plants are also a rich source of antibacterial and antifungal agents. These plants were selected to evaluate the possibility of new fungicides. But there were results where these plants couldn't be seen effective against plant pathogenic fungi. These plants were also tested in synergism to see the possibility of finding such a combination which can prove effective against fungi. Therefore, in the present study individual and synergistic effects of plant extract were determined against different plant pathogenic fungi.

Synergism of plant extract against fungi

In phytotherapy, there are potentially significant advantages associated with the synergistic interaction. Research on the synergistic effect of plant extracts on fungi as well as bacteria is very limited. According to our review there is no research reported on the synergistic effect of *A. racemosus* and *B. diffusa* against fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus* and *Fusarium moniliforme)* Synergistic effect of *Asparagus racemosus* and *Boerhaavia diffusa* against *Aspergillus fumigatus* is being reported for the first time. According to the observation of the present work combined stem extract of both the plant shows positive synergism by exhibiting highest inhibitory effect in comparison to the individual extracts. Combination of methanolic stem extract of *A. racemosus and B. diffusa* showed positive synergism against *Aspergillus fumigatus.* For the present study, fungal cultures were maintained on Sabouraud dextrose agar (SDA). Extraction of various plant parts extracted for screening against test fungi was done manually using mortar pestle and powder was obtained from dried plant parts using a grinder. Using mortar pestle, for individual plant extract of different plant parts i.e root stem and leaves of *Asparagus racemosus* and *Boerhaavia diffusa* 2gm powder of plant part was dissolved in 20 m distilled water and ethanol respectively. Combined plant extract 2 gm (1 gm of *Asparagus racemosus* parts and + 1 gm of *Boerhaavia diffusa* parts) were dissolved in 20 ml methanol. Maximum antifungal activity of combined plant parts of *Asparagus racemosus* and *Boerhaavia diffusa* against *Aspergillus fumigatus* was observed in methanolic extract of stem whereas no zone of inhibition was observed in root and leaves. Maximum antifungal activity of *Asparagus racemosus* was obtained against *Aspergillus fumigatus* in aqueous extract of stem and no zone was observed in leaves and roots. Maximum antifungal activity of *Boerhaavia diffusa* was obtained against *Aspergillus fumigatus* in aqueous extract of stem and no zone was observed in leaves and root. No zone of inhibition was found in root, stem and leaf extract of *Boerhaavia diffusa* and *Asparagus racemosus* against *Fusarium moniliforme, Aspergillus niger, Aspergillus terreus.*

L.S. Patel and R.S. Patel in (2013) reported maximum antifungal activity in root extracts. B Uma *et al.,* (2009) reported high degree of activity against all the *Candida* strains against root and tuber extract of *Asparagus*

racemosus. However, in the present study only aqueous extracts of the stem of *Asparagus racemosus* showed moderate inhibition against *Aspergillus fumigatus.*

This was quite different from the earlier reports as neither the aqueous nor ethanolic extract of root and leaves of *B.diffusa* and *A.racemosus* showed any positive results. . Agarwal *et. al.*, (2003) reported that extracts of aerial of *B.diffusa* did not show any noticeable antifungal activity. However aqueous extracts of the stem of *Boerhaavia diffusa* showed a high zone of inhibition against *Aspergillus fumigatus.* Combined extracts of roots and leaves of the 2 plants did not seem to be effective against *Aspergillus fumigatus.* However, methanolic extracts of combined stem extracts of *Boerhaavia diffusa* and *Asparagus racemosus* were highly effective against *Aspergillus fumigatus.*

Present study revealed that combination of both the plants in methanolic solvent gave good results. The combination of both the plants in the methanolic solvent stem was seen to be most effective against *Aspergillus fumigatus.* No such result about synergistic effects of these plants *Boerhaavia diffusa* and *Asparagus racemosus* against fungal pathogens have been reported yet and this seems to be the 1st report.

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