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Antifungal activity of *Ageratum conyzoides* Linn. leaf extract against *Aspergillus niger*

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ABSTRACT

Ageratum conyzoides Linn. is an aromatic plant belonging to family Asteraceae having potential of antifungal activity. In Well diffusion method, methanol extract of leaves of *Ageratum conyzoides* L. has shown a striking zone of inhibition is an average 16 mm against *Aspergillus niger*. The least minimum inhibitory concentration (MIC) was found out of plant extract *Ageratum conyzoides* L. with optical density 0.557 in 0.2 ml dilution. This study thus confirmed the antifungal activity or fungitoxicant potential ofAgeratum *conyzoides* L.

KEY WORDS:*Ageratum conyzoides L.*, *Aspergillus niger*, Antifungal activity.

INTRODUCTION

Fungi are widespread in the environment and fungal infections have become more frequent. Most of the fungi are responsible for yield losses while others spoil crops by producing potent toxins in crop plants and affect the quality of crop yield. Some individuals of fungi cause strong and dangerous allergic reactions (Boundless, 2016). The Food and Agriculture Organization stated that pests and diseases are responsible for about 25% of crop loss. Aspergillus is one of the most commonly involved in crop loss (Martinez, 2012. The number plants and animals have been affected by variety of fungal pathogen. Fugal pathogens have some harmful factors that allow them to reason for devastating damage to agriculture; affecting significant harvest losses of about 10% of harvest that threaten global food security.

In recent year, the world has facing some serious problem like contamination in stored commodities by fungi. In herbal and food industry having great concern of contamination by fungi and their mycotoxins. The genus of Aspergillus and Penicillin has reported for mycotoxins production in storage (Gautam and Bhadauria, 2009). By this results, the quality of food products and effects on the medicinal potential of herb.During storage of herbal drugs, *Aspergillus niger* or black moldwas recorded as a most dominating fungal species (Bugno, et. al., 2006). *A. niger* is a filamentous and saprophytic fungus found in soil, organic debris forage, and food product, causing black mould of onion, shallot; root stalk rot of Sanseveiria stem rot of Dracaena; and boll rot of Cotton; spoilage of cashew kernels, dried prune, dates, figs, vanilla pods (Bobbarala, et. al. 2009). The fungi *A. niger* cause food poisoning by aflatoxin production and it can also cause aspergillosis, a severe infection, in humans (Zhirongs, 1999).

In dairy industry, fungi are one of the main contaminants in dairy products, which provide a favorable place for their growth. Fungi are mainly responsible for visible or non-visible defects, such as flavor, off-odor and lead to significant food waste and economic losses. To prevent and control contamination, there are several traditional methods is called as called traditional hurdle technology. In prevention methods, air filtration, decontamination systems, good manufacturing and hygiene practices while control methods include temperature control, modified atmosphere packaging and inactivation treatments (Garnier, et al. 2017).

Roy (2022) investigated that, circulating paper currency notes in the local transport system in the city of Kolkata, is the one of the main source of fungal contamination which is harmful for human health. Wuyep et al. (2017) suggested that, plant *Ageratum conyzoides* Linn having some antifungal compounds which are very effective on various infections and diseases. They perform an antifungal activity *in vitro* and found that the aqueous and ethanolic extracts of. inhibited the growth of *Aspergillu*s *fumigatus, A. niger, A. tamari, A. terreus* and *A. ustus*.

Yusnawan et al. (2018) investigated that crude extracts of *Ageratum conyzoides, Amaranthusspinosus*, and *Cyperus rotundus* were used to suppress the rust disease intensity on Bima peanut cultivar. Some bacterial and fungal pathogen are inhibited by some chemical compounds extracted from *Ageratum conyzoides* L. (Dayie, Newman, Ayitey-Smith, & Tayman, 2007; Kamboj & Saluja, 2008). To control pea nut rust disease, concentrations of 2.5 % to 5 % of *Ageratum* extracts can be used.

Toan et. al (2019) shown that 6% leaf extract concentration of*Ageratum* effectively inhibits the rot disease caused by *A. niger* on chilli fruits. The leaf extract greatly inhibits fungal growth in vitro as well as isolates lesions, therefore check the rot on chilli fruits surface developes slowly. Nguyen et. al. (2021) isolated and identified antifungal compounds from the aerial parts of *Ageratum conyzoides* L. In field trial tests in a shaded net-house, the ethanolic extract on rice for blast disease is effective.

Awasthi et. al. (2010) studied eight commonly used spices such as *Piper nigrumSyzygium aromaticum, Zingiber officinale, Cinnamonum zeylanicum, Murraya koenigii, Allium sativum, Trachyspermum ammi* and *Allium cepa* for in vitro antifungal activity on *Aspergillus niger*. Five spices showed significant antifungal activity against the test pathogen. *Allium sativum* and *Syzygium aromaticum* and showed 100% inhibition of mycelial growth at 20% concentration. From this results, it is indicating that spices possess antifungal activity and can be exploited to control the growth of storage or spoilage fungi like *A. niger* and consequently reduce the dependency on the imitated fungicides.

Mandpe et al. (2016) investigated that *Launaea procumbens* of Asteraceae family is an aromatic plant has potential of antifungal activity. Methanol extract of leaves of *Launaea procumbens* has shown a striking zone of inhibition against *Aspergillus niger.*

Considering tremendous ability of plants for production of phytochemical compound, *Ageratum conyzoides* having antimicrobial drugs with reference to antifungal agent, a systematic investigation was undertaken to screen the local flora for antifungal activity from *Ageratum conyzoides*.

MATERIALS AND METHODS

Preparation of Plant Extract

Ageratum conyzoides was collected from garden of Botany Department, Campus, RTM Nagpur University. Matured leaves and young flowering shoots were thoroughly washed in running water and kept in shade dry. Dry materials were then ground finely by a blender and stored in zip lock polythene bags. Methanol was used as a solvent for extraction. 5g of powdered plant material was extracted by maceration with 20ml methanol and shaken on an orbital shaker for 48 hours.

Antifungal Activity

1. Well Diffusion Method

Weighted 200gm of peeled and chopped potato tuber transfer into the sterile conical flask containing 1000ml distilled water. After boiling, the supernatant was collected. Dextrose (20g) and agar (20g) was added and dissolved. Finally, the medium was transferred into measuring cylinder of 1-liter capacity and made the volume to 1 liter by adding more distilled water into it. The medium was poured into two or more Erlenmeyer flasks, cotton plug was put and the plugs were covered with aluminum foil and were autoclaved at 121˚C for 20 minutes. The flasks were taken out when temperature cools down and were used for antifungal activity. Agar well diffusion method was chosen forantifungal activity (Perez *et al.,1990).* The methanol and seven different plant extracts were tested against fungus *Aspergillus niger*. We used the method suggested by (Ambikapathy *et al., 2011*)with slight modification. After Potato Dextrose Agar (PDA) media was prepared, we added fungal culture in it and pour it in the sterilized petri plates and allowed to solidify. Next day, wells (6 mm) were made in the medium using sterile cork borer. 200µl of each extracts were transferred into the separate wells. The plates were incubated at 27˚C for 24 hrs. After the incubation, the plates were detected for formation of clear inhibition zone around the well point out the presence of antifungal activity. The zone of inhibition was recorded. *Griseofulvin* was used as a standard.

2. Minimum Inhibitory Concentration (MIC)

50 ml nutrient broth was taken in a sterilized test tube to make the fungal broth by inoculating the fresh fungal strain in it and kept at room temperature for 24 hrs. for incubation. Five sterilized test tubes of each plant extract were taken. The solution was prepared by adding 4 ml nutrient broth in each test tube. First test tube filled with 4 ml plant extract and shaken well. Then 4 ml solution transferred from first test tube to the second one and subsequently transferred in rest of the tubes for concentrations of 0.4, 0.2, 0.1, 0.05, 0.025 mg/ml. Each tube was then inoculated with 50 µl of fungal strain and incubated at 37˚C for 48 hours. The tubes were examined for visual turbidity. The turbidity was measured in terms of optical density (OD) at 610 nm by spectrophotometer. The MIC values were taken as the lowest concentration that inhibited the visual growth of the *Ageratum conyzoides*.

Result and Discussion

Table 1: Effect of Antifungal Activity of leaf extract of *Ageratum conyzoides* against *A. niger*

Sample Name	Zone of Inhibition(mm)			Mean (mm)
	Z_1	Z_2	Z_3	
Griseofulvin	12	12	12	12
Ageratum conyzoides	15	19	14	16

Table 2: Minimum inhibitory concentration (MIC) of methanolic leaf extract of *Ageratum conyzoides* against *Aspergillus niger*

Plants	Absorbance in different Concentrations(mg/ml)				
	0.4	0.2	0.1	0.05	0.025
Griseofulvin	0.847	0.247	0.154	0.618	1.713
Ageratum conyzoides	0.970	0.557	0.849	0.760	1.493

The zone of inhibition in methanolic extract of *Ageratum conyzoides* exhibited maximum antifungal activity (15, 19 and 14mm) than the standard *Griseofulvin* (12, 12 and 12mm). Therefore, methanolic leaf extract of *Ageratum conyzoides* showed significant antifungal activity than the standard used, against *Aspergillus niger* (Table 1). MIC value is used to evaluate antimicrobial nature of plant extracts and minimum quantity of antimicrobial compound required to kill or arrest multiplication of all microorganisms present in the medium or body fluid. The result of this investigation may open important perspectives in alternative antifungal therapies. Percentage growth inhibition was, calculated only with the finding of 4 ml of each concentration, which indicated that the values of optical density were further decreased by reducing visual growth or turbidity of fungal strain, shown in Table 2 and inhibition increases with decrease in concentration of plant extract. The least minimum inhibitory concentration (MIC) was found out of plant extract *Ageratum conyzoides* with optical density 0.557 in 0.2ml dilution. In the present study, the antifungal activity of a new combination was studied by micro dilution assay against *A. niger*.

Conclusion

The present study investigated that the methanolic leaf extract of *Ageratum conyzoides* from Asteraceae family possess some important chemical compound that can be perform antifungal activity and it may lead towards the novel discovery of antifungal drugs. It could be used mainly against the fungi *Aspergillus niger* to control the infectious diseases aspergillosis. In conclusion, the findings of this experiment confirmed that plant extracts can be used as natural fungitoxicant to control the growth of pathogenic fungi (*A. niger*).

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