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SCREENING OF SUCCESSIVE EXTRACTS OF *HAMELIA PATENS* FOR ANTIFUNGAL ACTIVITY

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Hamelia patens plant antimicrobial activity with various successive extracts of leaf is screening. Crude extracts of methanol, Ethyl acetate, Benzene and Hexane of leaf is evaluated for antimicrobial activity by the disc diffusion method. The term of medicinal, plants comprise a variety of types of plants used in herbarium and several plants have medicinal activities. The plant was selected for screening of antimicrobial efficacy against seven selected bacterial strains viz. *A.niger* (MTCC- 4325), *A.flavus* (MTCC- 3518), *A. terreus* (MTCC- 4287), *Penicillium chrysogenum* (MTCC- 2539), *Penicillium notatum* (MTCC- 6477). Pet ether leaf extract (75mg/disc) posses maximum efficacy against The antifungal activity maximum against *Aspergillus flavus* (Inhibition zone = 13mm, Activity index=0.81) and *Aspergillus terreus* (Inhibition zone = 10mm, Activity index=0.58).The results of this study show maximum efficacy against various fungal strains, these compounds represent the novel leads and the future studies and analysis may allow the development of a pharmacologically acceptable pure compounds or a class of antifungal agents. Therefore, the extract can also be used for isolation of volatiles compounds with potentials so that the extract / active fraction / pure compounds can be used as antibiotics in future therapeutics.

Keywords: Antibiotic; Antifungal activity; *Hamelia patens*; Pure compounds; Therapeutics.

Introduction

Infectious diseases represent a serious problem to health and they are one of the major causes of morbidity and mortality worldwide (World Health Organization 1998). Despite the significant advancement in human medicine, infectious diseases caused by microorganisms such as fungi are still a major risk to public health. The impact is still additional in developing countries due to the unavailability of medicine and the emergence of widespread drug resistance[1]. Infections induced by pathogenic fungi are increasingly recognized as an emerging hazard to public health[2, 3]. The increase in occur hence of fungal infections during topical years is due to a enlargement in the immune compromised population, such as organ transplant recipients and cancer and HIV patients[4]. Certain communal fungi, such as Candida species, cause infections when their human

hosts grow to be immune compromise[5]. These problems are also associated with resistance to antibiotics and toxicity during expanded treatment with numerous antifungal drugs[4]. In addition, the low efficacy, and side-effects and resistance associated with the existing drugs, emphasize the advent of safe, novel, and effective antifungal drugs. Plants fabricate a great deal of secondary metabolites, many of them with antifungal activity. Similarly, conventional medicine has made use of many diverse plant extracts for action of fungal infection and many of these have been tested for in vitro antifungal activity. Based on the facts that plants expand their own defense against fungal pathogens[6], they appear as an interesting resource for antifungal compounds.

Hence, there is a enormous demand for novel antifungal belonging to a broad range of structural classes, selectively acting on ne targets with fewer side effect[7]. One approach might be the testing of plants conventionally used for their antifungal activities as potential sources for drug development. Medicinal plants were not only significant to the millions of people for whom traditional drug is the only opportunity for health care and to those who use plants for a variety of principles in their daily lives, but also as basis of novel pharmaceuticals. Natural products, either as pure compounds or as standardized plant extracts supply unlimited opportunities for innovative drug showed the way because of the matched less availability of chemical assortment.

The results of this study show maximum efficacy against various fungal strains, these compounds represent the novel leads and the future studies and analysis may allow the development of a pharmacologically acceptable pure compounds or a class of antifungal agents[8].

Material and method

Collection-

The leaves of *Hamelia patens* was collected from sitapura,Jaipur, Rajasthan (India). This sample were authenticated and was given identification number and submitted in Ethno-medicinal Herbarium, Centre with potentials of Excellence funded by DST, JECRC University, Jaipur, India. Further, voucher specimens of *Hamelia patens* was deposited at herbarium of University of Rajasthan, Jaipur, India and was verified by senior taxonomist of department and provided with accession no. RUBL 206311.

Preparation of extracts-

Plant materials was collected, and then dried in shade. After complete dry, fine powder was made by pestle mortar. Powder sample was refluxed for 72h at room temperature. The extracts were added into clean petriplate for evaporation then allowed for evaporation. The extracts were collected in screw capped bottles. The extracts were used for antifungal activity. The extracts were stored at 20°c for experimental use. Bioefficacy of the extract was checked *in vitro* by well in agar diffusion method[9].

Activation of fungi

Loopful fungal spores were streaked on potato dextrose agar (Hi- media) plates and incubated at 37°c for 2-3 days. All fungus plates were maintained at 4°c in refrigerator for further use.

Zone of Inhibition

For determination of zone of inhibition, basically three methods are used. One of them is a well diffusion method which we have used.

A. Preparation of potato dextrose agar medium (PDA agar medium)

Preparation of PDA includes the following steps

i. PDA agar medium was prepared from commercially available dehydrated base according to the manufacturer instructions.
ii. Immediately after autoclaving, allowed to cool in 45 to 50°c water bath.
iii. The freshly prepared and cooled medium was poured into the glass or plastic flat bottomed petri dishes till the level, horizontal surface to give uniform depth.
iv. The PDA agar medium should be allowed to cool at room temperature and until the use plates were stored in a refrigerator.
v. Plates should be used within 7 days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of agar.
vi. Representative samples of each batch of plates were examined for sterility by incubating at 30-35°c for 24 hours.

Preparation of disc

The discs were made from wattamn filter paper using the punching machine. The discs were deeped into different plant extract concentration. Plates were used for the zone of inhibition test.

Procedure for performing the well diffusion method

Inoculums preparation

Three to five well-isolated colonies of the fungus were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4-5 ml of PDA broth medium. The broth culture is incubated at 350 C until it achieves turbidity 1-2 x 108 CFU/ml. The turbidity of actively growing broth culture was adjusted with sterile saline.

Inoculum of test plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, loopful of suspension inoculates into flask contains Agar and added the watman paper discs. Mix it well and pour it into plate and rotate the plate for even distribution. On the dry PDA agar plate loopful suspension evenly spreaded by spreader[10].

Preparation of disc of plant extract

i.In the plate, discs were made for the inoculation of plant extract. Minimum four discs were put in one plate.

ii.Using micropipette, 30µl of antifungal drug was added and extracts into respective disc[11].

iii.The plates were first placed at 4°c for 30 min in order to diffusion of extract and antifungal drug.

iv.Then plates were incubated at 37°c for 24 hours at room temperature.

v.The diameter of the inhibition zones were measured in millimeter at the end of the incubation time.

Procedure for performing the minimum inhibitory concentration Test inoculums

i. Different concentration of plant extract discs in (25ml, 50ml and 75ml) to the tube to respective discs was added.

ii. From the inoculums 25ml of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106 cells/ml. This procedure was performed for all the positive extracts antifungal activity which were obtained by primary screening.

iii. Then all sets of disc of dilution broth were incubated at 37°c for 24 hours in incubator[12].

All sets of tubes were observed for determination of MIC to the susceptible fungus were tested and note down the results.

Results and Discussion

Antifungal activity of *Hamelia patens* against fungal species: When antifungal of

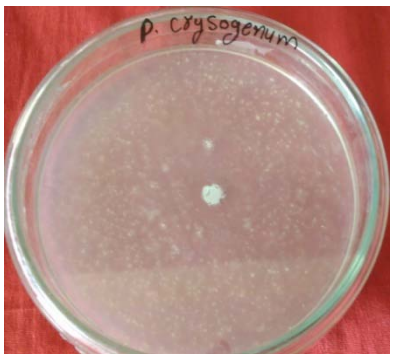


Fig. 1. *P. crysogenum*

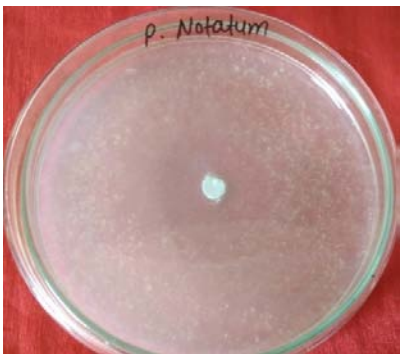


Fig. 2. *P. notatum*

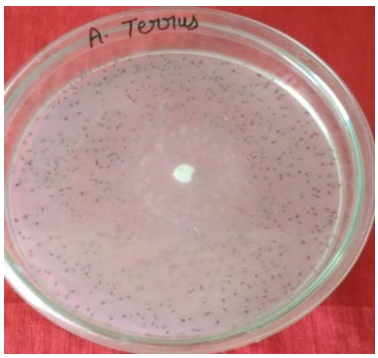


Fig. 3. *A. terreus*

Fig. 4

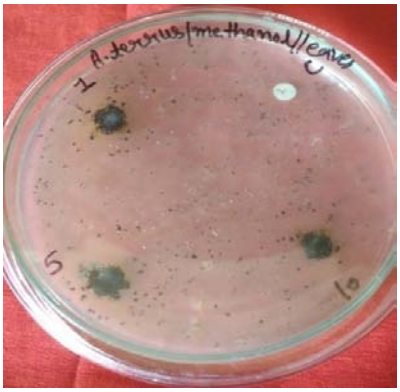


Fig. 5

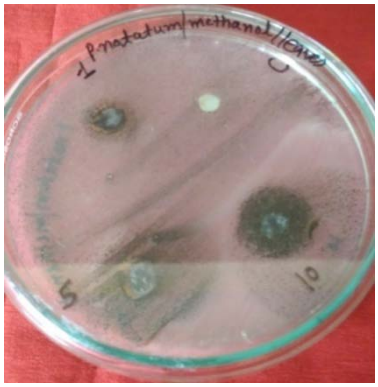


Fig. 6

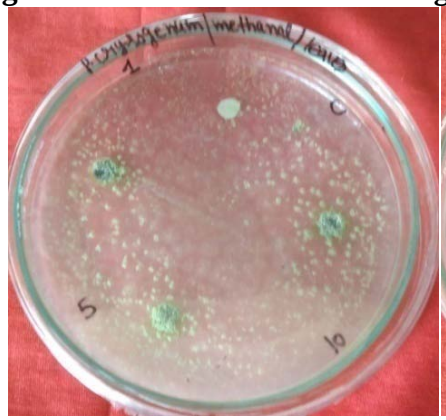


Fig. 7

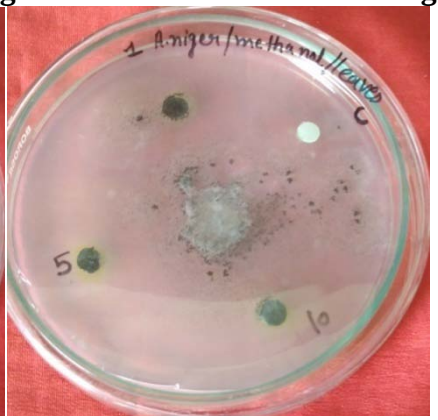


Fig. 8

Hamelia patens leaf was performed against 5 fungal flora through the preparation of **Methanol leaf** extract of plant and disc of 25 mg/ml, 50 mg/ml, 75 mg/ml was prepared the results of *Hamelia patens* activity were appreciable. The antifungal activity maximum against *Aspergillus niger* (Inhibition zone = 4mm, Activity index = 0.25) and *Penicillium notatum* (Inhibition zone = 12mm, Activity index=0.66).

- A=25mg/disc, B=50 mg/disc, C=75 mg/disc
- IZ = Inhibition zone, A.I. = Activity index

Antifungal activity of *Hamelia patens* against fungal species: When antifungal of *Hamelia patens* leaf was performed against

Table 1. Showing the inhibition zone and activity index of methanol leaf extract of *Hamelia patens* against test microorganism.

Fungus	IZ of standard	Inhibition zone					
		(A) 25 mg/disc		(B) 50 mg/disc		(C) 75mg/disc	
		IZ	AI	IZ	AI	IZ	AI
A.niger	16	3	0.18	4	0.25	4	0.25
A.flavus	19	0	0	0	0	0	0
A.terreus	17	2	0.11	4	0.23	5	0.29
P. chrysogenum	15	0	0	0	0	0	0
Penicillium notatum	18	5	0.27	10	0.55	12	0.66

Table 4. Showing the inhibition zone and activity index of **Pet ether** leaf extract of *Hamelia patens* against test microorganisms.

Microorganisms	IZ of standard	Inhibition zone					
		(A) 25 mg/disc		(B) 50 mg/disc		(C) 75mg/disc	
		IZ	AI	IZ	AI	IZ	AI
A.niger	18	2	0.11	2	0.11	3	0.16
A.flavus	16	3	0.18	4	0.25	5	0.31
A.terreus	17	2	0.11	0	0	3	0.17
p. chrysogenum	19	3	0.15	4	0.21	4	0.21
penicillium notatum	12	2	0.16	4	0.33	7	0.58

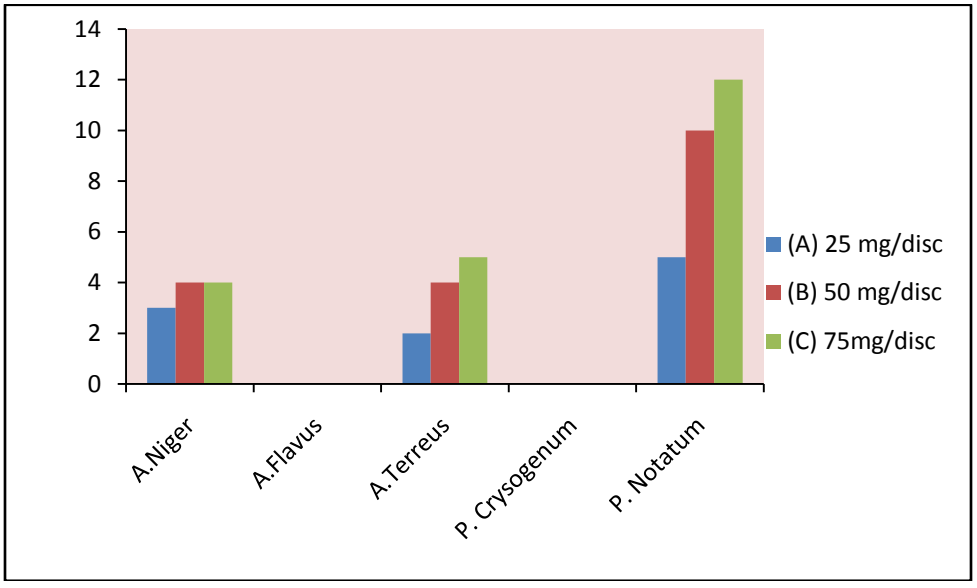


Fig. 9 : Graphical representation of antifungal activity of *Hamelia patens* methanol leaf extract

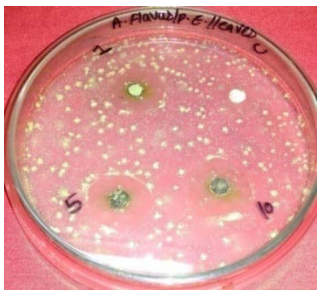
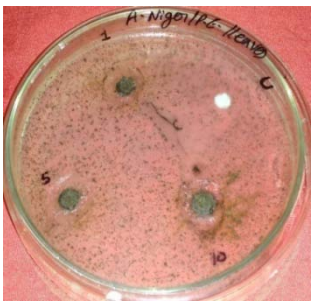


Fig. 10



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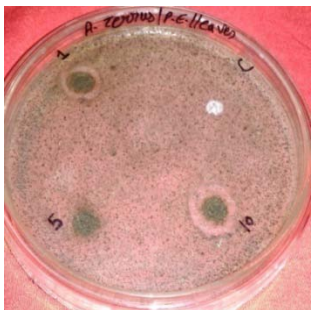

Fig. 12

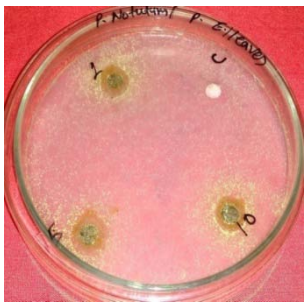

Fig. 13

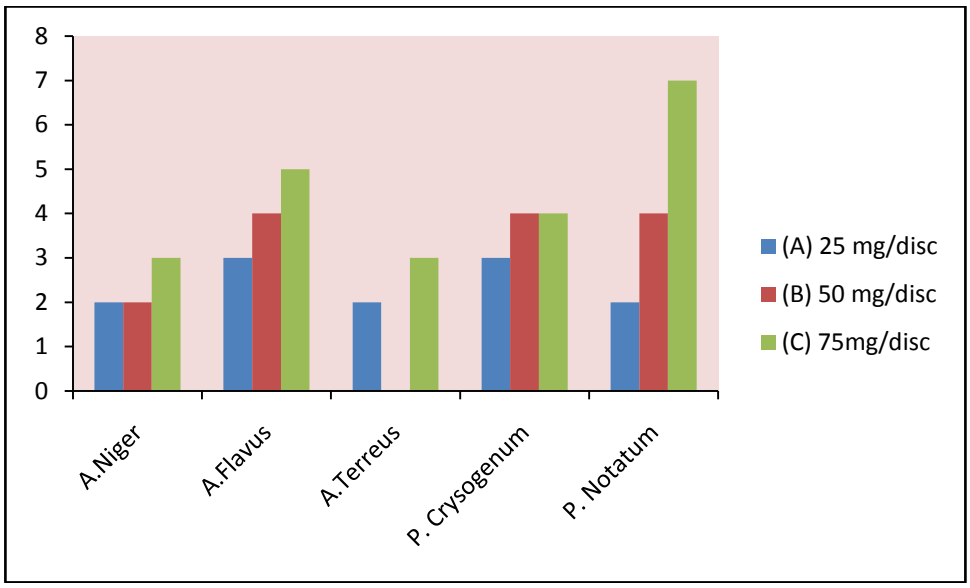

Fig. 14. Graphical representation of antifungal activity of *Hamelia patens* Pet ether leaf extract

5 fungal flora through the preparation of **Pet ether** extract of plant and disc of 25 mg/ml, 50 mg/ml, 75 mg/ml was prepared the results of *Hamelia patens* activity were appreciable. The antifungal activity maximum against *Aspergillus flavus* (Inhibition zone = 5mm, Activity index=0.31) and *Penicillium notatum* (Inhibition zone = 07mm, Activity index=0.58).

Antifungal properties of medicinal plants are being increasingly reported from different parts of the world[13]. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population[14].

The above results show that the activity of methanol and pet ether extracts of *Hamelia patens* shows significant antifungal activities. This study also shows the presence of different phytochemicals with biological activity that can be of important therapeutic index. The result of phytochemicals in the current investigationshowed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids[15]. Results show that plant rich in tannin and phenolic compounds have

been shown to posses antimicrobial activities against a number of fungal species.

The results obtained from this work showed that plant extracts antifungal effects maximum against *Aspergillus niger, Aspergillus flavus* and *penicillium notatum.* These 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[16]. 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.

Conclusion

The use of natural products as alternative agents for the control of fungal diseases is considered as an interesting alternative to synthetic fungicides[17]**.** The result obtained in this study clearly demonstrates broad-spectrum antifungal activity. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents[18]. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds[19]. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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