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THE STUDY WAS AIMED TO DETERMINE THE ANTIFUNGAL ACTIVITY OF ALLIUM SATIVUM (GARLIC) ON SOME SELECTED FUNGI

Datta Mane*[1] and Bhalchandra Wadikar[2]

Assistant Professor, S.V.E.T'S College of Pharmacy, Humnabad.

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***Corresponding Author**
Datta Mane
Assistant Professor,
S.V.E.T'S College of
Pharmacy, Humnabad.

ABSTRACT

Garlic samples were purchased from Humnabad local market. The samples were washed, separated and peeled to obtain the edible portion. The fungi were isolated using the culture method and identified based on morphological characteristics. The extract was prepared using two solvents (aqueous and ethanol) by soaking method. The antifungal activity of aqueous and ethanolic garlic extract was determined on some selected fungi namely, Fusarium spp and Rhizopus spp. From the results it is clear that, ethanol extract showed more activity when compare to aqueous extract. The diameter of zones of inhibition for the ethanolic extract ranged between 4.1-14.3 mm, while that of aqueous extract ranged between 2.4-10.4 mm. The MIC for the ethanolic extract was 2.5 mg/ml and 5.0 mg/ml for Fusarium spp and Rhizopus spp respectively. While for aqueous extract there was no effect on both tested organisms. It can be concluded from this study that garlic extract showed antifungal activity against the test organism. Moreover, the ethanolic extract showed inhibitory activity among the tested fungi.

KEYWORDS: Photochemical, ethanol, garlic**,** growth (+), no growth (-).

INTRODUCTION

The medicinal and antimicrobial activities of extracts from plants are gaining attention of researchers worldwide. The modern medicine has its own advantages and side effects, so the plant based products are getting more popularity, as they are safe to use, and comparatively easily available and cheap. Many extracts possess antifungal activity. Plant extracts and

essential oils are effective in plant pathogens. Apart from the use of plant based products in medicine, the usage of these extracts in plant protection also now becoming popular throughout the world. Garlic is one among the important earliest known medicinal plants. Its usage worldwide has a long history. Being an important food spice plant, it has significant role in disease prevention and control, many of the diseases can be cured with garlic. It has been used since long time against human pathogens. But studies are less regarding the usage of garlic against plant pathogens. Some earlier works deals with the action of garlic against pathogens. The aim of this research work was to determine antifungal activity of aqueous and ethanolic garlic extract on some selected fungi at different concentrations and to determine the minimum inhibitory concentration (MIC) of aqueous and ethanolic extract of garlic. This systematic review, synthesizing data from 434 published papers, estimates that ~60 million Indians (4.1%) are afflicted by a serious fungal disease. Tinea capitis and recurrent VVC account for 49 million, with chronic pulmonary aspergillosis & fungal rhinosinusitis affect over 3 million. Globally, fungal diseases are known to impact millions of lives. However, the epidemiology of fungal infections varies in different geographical regions, being dependent on multiple factors including at-risk individuals, socioeconomic attributes, and fungal endemicity related to geo-ecological characteristics, all leading to substantial impacts on health. India is the second most populous country in the world and the seventh largest country by land area. This tropical country has unique and diverse geographical characteristics, with mountains, plains, plateaus, and numerous rivers, in addition to being surrounded on three sides by vast stretches of ocean. Many fungal infections are endemic in India, and several pathogens found globally are frequently isolated there. The presence of fungal disease has been implicated from ancient times in the Indian subcontinent; the Atharva Veda, an ancient Hindu scripture written between 1500 and 500 BCE, mentions mycetoma (“pada valmikam,” or “anthill foot)” in the Indian population. The first case of histoplasmosis was reported in Calcutta in 1954, followed by gradual recognition of the impact of other fungal diseases in this population. Several host characteristics, such as a very high incidence of pulmonary tuberculosis (PTB), malignancies, chronic pulmonary obstructive disease (COPD), and others, also contribute to a high predilection for fungal diseases in the Indian population. Many outbreaks of different fungal infections have been periodically reported from different parts of India, including Candida auris and, more recently, COVID-19- associated mucormycosis.

Morphological characteristics

Colour: Bulbs are white to pink in colour

Odour: Characteristics and aromatic

Taste: Aromatic and pungent

Size: 1.5 to 2.5 cm

Chemical constituents

Garlic bulbs contain 29 percent of carbohydrates, about 56 percent of proteins (Albumin), 0.1 percent of Fat, Mucilage, and 0.06 to 0.1 percent of volatile oil. It also contains phosphorus, Iron, and copper.

Volatile oil of the drug is the chief active constituent and contains Allyl propyl disulphide, diallyl disulphide, alliin, and allicin.

USES

Garlic is used as carminative, aphrodisiac, expectorant, stimulant and disinfectant in the treatment of pulmonary conditions.

It is largely used as condiment. oil of Garlic is used as

Anthelmintic and rubefacient.

Allicin is antibacterial. Garlic oil is use full in high blood pressure and atherosclerosis.

Fresh Garlic is prophylactic against amoebic dysentery.

It has strong antioxidant effect.

MATERIAL AND METHODS

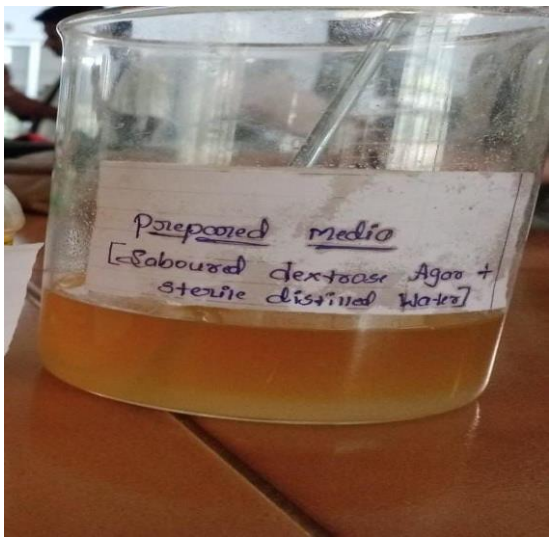
Materials Following materials used in Research work Of antifungal activity of garlic extract on some selected Fungi of grade or best possible pharma grade available were used as supplied by the manufacture.

LIST OF MATERIALS

1) Garlic
2) Sabouraud dextrose agar(SDA)
3) Ethanol
4) Weighing machine
5) Conical flask
6) Distilled water
7) Hot plate
8) Autoclave
9) Petri dish
10) Rhizopus SPP and fusarium SPPas bactericidal, antifungal and antiviral actions

Method of preparation of agar media

There are various methods are available to prepare agar media. In that we used disc diffusion method to Prepare agar media.



DISC DIFFUSION METHOD

To prepare agar medium for the disc diffusion method following steps are used.

1) ***Ingredients***: *Sabouraud dextrose agar *Nutrient broth or another appropriate liquid medium, Distilled water.

Steps for preparation

* Weigh about 19.5grams of sabouraud dextrose agar by using weighing machine.
* Dissolve the weighed agar in the conical flask containing 300ml of sterile water.
* It was shaken to mixup and dissolve in a hot plate.
* Sterilize the medium by autoclaving. Follow the standard autoclaving protocols, typically 15 minutes at 121*c
* Allow the medium to cool to around 45 to 50*c before pouring in to petri dishes
* Pour the sterilized and cooled agar medium in to petri dishes to solidify. Avoid creating bubbles.
* Allow the plates to cool and dry with their lids slightly ajar to prevent condensation.
* Ensure the sterility by inspecting for contamination before us. * Store the prepared agar plates at a specific period of time.

Preparation of a fungal culture media

The fungus called Rhizopus SPP and Fusarium SPP is collected from a veterinary college and it can be sub-cultured before transferring it to the culture media in an aseptic area with the help of flaming.

And the culture media is kept in a BOD incubator at a 15 to 25*C in a specific period of a time. After 2-3 days the fungal culture media is prepared.

Confirmation of fungal media

For the confirmation of fungal media we used Lactophenol cotton blue staining method.

Lactophenol cotton blue staining

Materials required

Slide, cover slip, lactophenol cotton blue stain, "L" shaped loop colony of fungi.

Procedure

1. With the help of "L" shaped loop takes small colony quantity of the growth on the slide. Dissect it well.
2. Put 2 to 3 drops of LCB stain with cover slip
3. Observe the morphology under low power and than under high power.

Preparation of a garlic extract

The garlic (Allium sativum) is collected from A Humnabad local market. And the peels are removed and remaining Portion are kept in a hot air oven at 45*C For 2 to 3 days to remove its moisture. And further dried garlic makes in to a fine Powder with the help of mortar pistil. The garlic contains a Allicin as a active Chemical constituent which is responsible for antifungal activity. For the test determination the garlic extract Powder placed in a fungal culture media and it is shows as a zone of inhibition as a result.

Zone of inhibition

In the determination of antifungal activity we used agar plate method to find out the minimum and maximum zone for the inhibition activity.

Agar plate method

The agar plate method is a common technique used to assess the effectiveness of antifungal agents. In this method, a petridish containing a solid agar medium is inoculated with bacteria, and paper discs infused with different antifungal substances are placed on the agar surface. After incubation, the zone of inhibition, where bacterial growth is inhibited around the disc, is measured to determine the agent's effectiveness against the tested microorganisms.

Table 1: Antifungal activity of ethanolic extract on the growth of Fusarium and Rhizopus spp.

Diameter of zones of inhibition against the		
Concentration	**Fusarium**	**Rhizopus test organism**
1.5	------	-----
2.5	4.1	5.2
5.0	6.2	6.4
10.0	10.1	9.1
control	14.3	12.2

Table 2: Minimum inhibitory concentration of ethanolic extract of garlic.

Test organism	**Concentration of extracts (mg/ml)**					
	1.5	**2.5**	**5**	**10**	**20**	**MIC**
Fusarium spp	+	-	-	-	-	2.5
Rhizopus spp	+	+	-	-	-	5

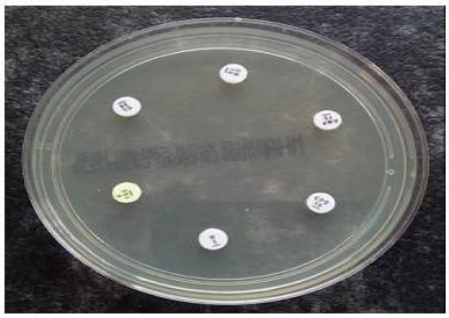
Table 3: Antifungal activity of aqueous extract on the growth of Fusarium and Rhizopus spp.

Diameter of zones of inhibition against the		
concentration	Fusarium	Rhizopus test organism
1.5	------	-----
2.5	-----	-----
5.0	2.4	4.3
10.0	4.2	5.2
20	9.5	10.4
control	-----	------

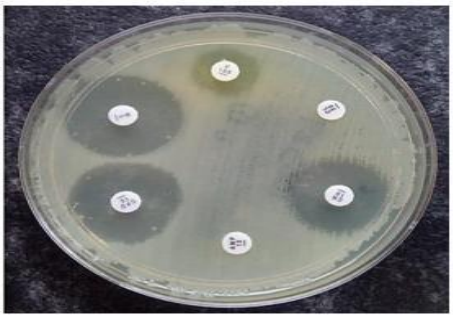
Key;- means no acitivity, the result showed minimum inhibitory concentration (MIC) of ethanol garlic extract. The MIC was found to be 2.5mg/ml for fusarium spp and 5.0mg/ml for rhizopus spp as presented in table no 2.

Table 4: Minimum inhibitory concentration of ethanolic extract of garlic.

Test organism	Concentration of extracts (mg/ml)				
	1.5	2.5	5	10	20
Fusarium spp	+	+	+	+	+
Rhizopus spp	+	+	+	+	+



(fig.13-a Disc formation)



(fig.13-b Initiation of formation of zone of inhibition)

(Fig.13-c zone of inhibition after some time)

RESULT

The results of antifungal activity obtained showed that the ethanolic plant extracts has no effect on all the test organisms at 1.5 mg/ml. However, at 2.5 mg/ml, 5.0 mg/ml, 10.0 mg/ml and 20.0 mg/ml, the plant extracts showed inhibition zones of 5.2, 6.4, 9.1 and 12.2 mm for Rhizopus spp. While at 2.5 mg/ml, 5.0 mg/ml, 10.0 mg/ml and 20.0 mg/ml, the plant extracts showed inhibition zones of 4.1, 6.2, 10.1 and 14.3 respectively for Fusarium spp. in which 20 mg/ml showed the highest zones of inhibition with inhibition of14.3 mm for Fusarium spp and 12.2 mm for Rhizopus spp., for the control, there was no effect as presented in table.

DESCUSSION

Garlic (Allium sativum) is a spice with global recognition. In the present study, it has been shown to inhibit the growth of fungi when tested. The antifungal action of garlic is due to the

compound allicin. Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi. Our results clearly indicate that the garlic ethanol extract showed higher inhibitory activity. Likewise, the aqueous extracts of garlic show less antifungal activity than the ethanolic extract against the test organisms, which is in agreement with earlier reports. Moreover, all the extracts of garlic in higher concentrations showed that the antifungal effect increase when the concentration is also increased.

Inhibition in growth of Fusarium spp and Rhizopus spp observed in this study was similar to previous findings of who demonstrated antifungal potency of garlic where inhibition of Trichophyton and Microsporum species using fresh garlic juice was shown due to stronger activity of ajoen.

CONCLUSION

It can be concluded from this study that garlic extracts showed antifungal activity against the test organisms. Moreover, the ethanolic extract showed inhibitory activity among the tested fungi namely Fusarium spp and Rhizopus spp, where Rhizopus spp is more susceptible to aqueous extract and Fusarium spp more susceptible to the ethanolic garlic extract.

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