



Antifungal Activities of *Tamarindus indica* and *Azadirachta indica* Extracts on the Growth of Some Selected Fungal Species

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ABSTRACT

Plants have provided a source of inspiration for novel drugs compounds, as plant derived medicines have made large contributions to human health and well-being. In present study, the chloroform extracts of *Tamarindus indica* and *Azadirachta indica* leaves were investigated for their antifungal activity against *Aspergillus niger* and *Aspergillus flavus* using the disc diffusion method in triplicate against the two pathogenic fungi at the concentrations of 150 µg/disc and 300 µg/disc for each. Results obtained showed that all the extracts reduced colony growth by 25-56%. It was found that among the leaf extracts tested, *A. indica* have maximum percentage growth inhibition (56%) against *A. flavus*, while *T. indica* extracts showed maximum percentage growth inhibition (27%) against *Aspergillus niger*. It can be used in the folk medicine at different parts of the world to treat many diseases including fungal infections. These promissory extracts open the possibility of finding new clinically effective antifungal compounds.

Keywords: Antifungal, *Aspergillus flavus*, *A. niger*, *Tamarindus* and *Azadirachta indica*

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries (Shehu *et al.* 2014). To overcome this problem many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs (Srinivasan, *et al.* 2001). Considerable research is prompted for development of antifungal agents with diverse mechanism of action due to continuing increase in incidences of life threatening and invasive fungal infections, their narrow activity spectrum, fungi static rather than fungicidal activity and resistance development in fungal species (Cavaleiro *et al.*, 2006).

Tamarindus indica (commonly called Tamarind), family Fabaceae, subfamily *Caesalpinaceae* is a tropical evergreen tree native to fertile areas throughout Africa and Southern Asia. It is widely cultivated as an ornamental tree and for its acidic fruits used in making drinks and a popular component of many decoctions used as health remedies. In Northern Nigeria, the fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser.

Neem (*Azadirachta indica*), a large tree of India, has been used for centuries in Asia as insecticides, fungicides, anticonceptionals in popular medicine almost every part of this tree seeds, leaves, roots, bark,

trunk and branches has multiple uses (Chaturvedi et al., 2003) and has been recommended to plant in African and Asia by many international organizations

Antifungal properties of *Tamarindus indica* and *Azadirachta indica* extract are also reported by some authors (Vaghasiya et al. 2008; Pathmanathan et al. 2010). Further screening of these medicinal plants may lead to discovery of antifungal compounds. Despite the great success of pharmaceutical industries in developing new antibiotics finding new broad spectrum antifungal agents is still a priority due to the resistance development of fungal strains as well as absence of licensed antifungal vaccines. Better activity of these extracts with standard fungicides proves the possible practical applicability of the extracts. Also these extracts are biodegradable, renewable in nature, safe to human as well as plant health and simple in application. Hence, the use of these extracts as broad spectrum fungicides without creating any health problems to animals and human beings has proven. In the present investigation different concentration of plant extract were evaluated for the antifungal potential against various pathogenic fungi.

MATERIALS AND METHODS

Collection of plant material

Young shoots of *Tamarindus indica* and *Azadirachta indica* were collected, washed and then sun-dried for four days. This leaves were then removed and placed on a clean sheet of old newspaper and then weighed. The weighed leaves were then pounded separately in a mortar before being transferred to the blender to get fine powders.

Plant Material Extraction

The powders were then sieved to get the fine and smooth powder. Their weights were taken and transferred to sterilized containers which are labelled. 50 g of the plant were extracted (cold) with chloroform (50cl) in flat bottom glass container, through occasional shaking and stirring for 15 days. The whole mixture was then filtered and the filtrate was dried in vacuum using a rotatory evaporator (Sudharameshwari et al. 2007) to afford a blackish mass.

Organisms Collection

Antifungal screening was carried out against two fungi: *Aspergillus niger* and *Aspergillus flavus*. These organisms were collected from the Mycology Laboratory of Biological Sciences Department, Usmanu Danfodiyo University, Sokoto.

Antifungal Screening

The antifungal activity of the extract was tested by disc diffusion method (Wei et al. 2006; Dash et al. 2005) against the two pathogenic fungi at the concentrations of 150 µg/disc and 300 µg/disc for each. Here 20 ml quantities of Sabouraud dextrose were plated in petri dish. Blank disc impregnated with solvent chloroform followed by drying off was used as negative control.

The activity was determined after 72 h of incubation at room temperature (32°C). The diameter of zone of inhibition produced by the extract was then compared with the standard antibiotic kanamycin 30 µg/disc. Each sample was used in triplicate for the determination of antifungal activity.

RESULTS AND DISCUSSION

The antifungal activities of chloroform extract of *Azadirachta* and standard kanamycin (30 µg/disc) were determined at the concentrations of 150 µg/disc and 300 µg/disc against two pathogenic fungi (Table 1). The highest activity was 19.0 mm diameter of zone inhibition observed against *Aspergillus flavus* followed by 17.0 mm diameter of zone inhibition against *Aspergillus niger* at the concentration of 300 µg/disc. On the left hand, the lowest activity was 8.0 mm diameter of zone inhibition found against *A. niger* at the concentration of 150 µg/disc, control remain at 25 mm for both fungal pathogens.

In *T. indica* (Table 2) the highest activity was 11.0 mm diameter of zone inhibition observed against *Aspergillus niger* followed by 9.0 mm diameter of zone inhibition against *Aspergillus flavus* at the concentration of 300 µg/disc. However, the lowest activity was 5.0 mm diameter of zone inhibition found against *A. flavus* at the concentration of 150 µg/disc, control inhibition covered 20.7 and 22.0 mm for *A. niger* and *A. flavus* respectively.

Table 1: Antifungal activity of chloroform extract of *Azadirachta indica*

Fungal pathogens	Diameter of zone of inhibition (mm)		
	Chloroform extract 150 µg/disc (M±SE)	Chloroform extract 300 µg/disc (M±SE)	Kanamycin 30 µg/disc (M±SE)
<i>Aspergillus niger</i>	8.0±0.6	17.0±1.2	25.0±0.0
<i>Aspergillus flavus</i>	9.0±0.6	19.0±1.0	25.0±0.5

Table 2: Antifungal activity of chloroform extract of *Tamarindus indica*

Fungal pathogens	Diameter of zone of inhibition (mm)		
	Chloroform extract 150 µg/disc (M±SE)	Chloroform extract 300 µg/disc (M±SE)	Kanamycin 30 µg/disc (M±SE)
<i>Aspergillus niger</i>	7.0±0.2	9.0±0.7	20.7±1.8
<i>Aspergillus flavus</i>	5.0±0.0	11.0±1.8	22.0±0.0

Different plant extracts have been reported for their antifungal properties (Wei *et al.* 2006; Dash *et al.* 2005; Owolabi, *et al.* 2007; Doughari, *et al.* 2008), which supports our present findings. Overall, the chloroform extract of *Azadirachta indica* showed significant activity against all the tested pathogenic fungi.

Several authors (Wei *et al.* 2006; Dash *et al.* 2005) had reported the fact that the extract of certain spices and herbs of medicinal importance exhibit antifungal property. These natural antifungal agents could be exploited in controlling the growth of fungi consequently inhibiting aflatoxin formation (Greyer and Harborne, 1994). All the extracts were found to inhibit the growth of selected fungi. The percentage growth inhibition of *A. flavus* was found maximum with high zone of inhibition. Efficacy of treatments increased with increased concentration of leaf powder used. There was a significant difference between the treatments at P=0.05

The present results of this investigation exhibits the radial growth of *Aspergillus* was inhibited by chloroform leaf extracts of *Azadirachta indica*, suggesting the presence of antifungal substances in the plant tissue, which agreed with the results reported by other workers on different pathogens and plants (Abed *et al.*, 1993, Qasem *et al.*, 1996; Amadioha 1998 and Amadioha 2003).

This may therefore explain the demonstration of antimicrobial activity by the leaf extracts of *Tamarindus indica*. The demonstration of antifungal activity against *Aspergillus* may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan *et al.* 2001). This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times.

CONCLUSION

In conclusion, different concentration of chloroform plant leaf extract showed antifungal activity. Among the plant tested, *A. indica* chloroform extract of leaves showed higher antifungal activity against *A. niger* and *A. flavus* than *T. indica* chloroform extract. Efficacy of treatments increased with increased concentration of extract used. There was a significant difference between the treatments. This gives an indication of the presence of promising antifungal compounds. Authors also designed further study of photochemical of selected plant to elucidate the components responsible for antifungal activity. These promissory extracts open the possibility of finding new clinically effective antifungal compounds.

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