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Comparative *in vitro* antifungal activities of *Simarouba glauca* against *Fusarium oxysporum* and *Aspergillus parasiticus*

Khaling Mikawlawng^{1*}, Sandeep Kaushik², Anand Kumar Pushker³, Suresh Kumar⁴, Moirangthem Kameshwar Singh⁵, Gurumayum Suraj Sharma⁶

1. Department of Botany, Ramjas College, University of Delhi, Delhi, India-110007
[Email: khaling55@gmail.com; Tel: +919953228994]
2. Department of Botany, Ramjas College, University of Delhi, Delhi, India-110007
3. Department of Botany, Ramjas College, University of Delhi, Delhi, India-110007
4. Department of Botany, Ramjas College, University of Delhi, Delhi, India-110007
5. Departments of Zoology, University of Delhi, Delhi, India-110007
6. Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, Delhi, India-110 007

Fusarium oxysporum and *Aspergillus parasiticus* cause diseases to animals including human directly or by producing mycotoxins. *A. parasiticus* is well known for its production of aflatoxin which is a potent carcinogenic compound. *F. oxysporum* is reported as a serious emerging trans-kingdom pathogen. Once established, fungal diseases caused by these fungi are difficult to treat and most of the available antifungal therapies are generally of limited value due to toxicity problems. The present study analyses the antifungal properties of *Simarouba glauca*, a medicinal plant well known for its antimicrobial, antidiarrheal, antiherpetic, antihelminthic and antiprotozoal activity. Methanolic and ethanolic extracts of both fresh and dried leaves were tested for their inhibitory activity against these pathogenic fungi. Screening of the crude extracts for the antifungal activity using well diffusion assay showed strong inhibition against the tested fungus. Ethanolic extracts of both the fresh and dried leaves were found to be more effective as compared to methanolic extracts against the growth of the fungi. The present study also showed that the leaf extracts of *Simarouba glauca* is more effective against *Aspergillus parasiticus* as compared to *Fusarium oxysporum*.

Keyword: Medicinal plants, *Simarouba glauca*, antifungal, *Fusarium oxysporum*, *Aspergillus parasiticus*

1. Introduction

Fungi comprise of varied collection of eukaryotic organisms that mostly live in dead and decaying organic matters. They attributed great benefit to human health in the form of fungal-derived antibiotics, such as penicillin, cephalosporin, enzyme production and food processing, and are indispensable for health and also play important role through their ability to break down complex organic matter and recycle essential nutrients back into the environment^[1-3]. However, despite their extensive influence on ecology, health and economic well-being, the threats posed by

emerging fungal pathogens are often unappreciated and poorly understood. Most fungi are saprophytes, whereas few fungal species are parasitic causing diseases in plants or animals. Around 5.1 million species of fungi are believed to exist on earth^[4], however from these only few are capable of causing diseases in humans and plants. Most fungal species can cause disease on a single host plant or animal and less fungi have the ability to infect both plants and animals. The mechanisms that control host range in fungi are not fully known. Though plants and animals represent two different host types, some fungi

have overcome this inter-kingdom bridge and evaded both plants and animals; such fungi are referred to as trans-kingdom pathogens [5]. Examples of such fungi include *Fusarium* and *Aspergillus*. *Fusarium* spp. can cause diseases in animals and humans when grains contaminated with *Fusarium*-derived T2 mycotoxin are ingested. This can lead to alimentary toxic aleukia characterised by gastrointestinal symptoms, weakness, myositis, aplastic anaemia, and even death. These fungi can even directly infect tissue surfaces and cause mycotic keratitis, endophthalmitis, or onychomycosis. Immunosuppressed patients have higher chance of developing hyalohyphomycosis, osteomyelitis/arthritis, or peritonitis during peritoneal dialysis. Such individuals are also highly prone to fatal systemic fusariosis during chemotherapy [6]. *Fusarium oxysporum* is a soil-borne fungus which causes vascular wilt in large variety of economically important crop plants [7]. *F. oxysporum* strain 4287 was reported to infect both plants and mammalian hosts [8]. Besides its well-studied activity as a plant pathogen for vascular wilt in important crop plants, soil-borne fungus *Fusarium oxysporum* is reported as a serious emerging pathogen of humans due to the increasing number of severe cases reported and to its ability to resist broad variety of currently available antifungal drugs [9, 10]. The diseases caused by most species of *Fusarium* are generally resistant to most of the available antifungal drugs [11].

Aspergillus parasiticus is a soil-borne fungi thriving on both living and decaying organic matter. It is also one of the aflatoxin producing fungi, the other genus being *Aspergillus flavus*. Various studies have reported the linked between liver cancer incidence and consumption of aflatoxin contaminated foods in the diet [12-14]. Aflatoxicosis (a terminology for food poisoning caused by ingestion of foods contaminated with aflatoxins) can result in direct liver damage and death. Thus, foods contaminated with these toxigenic fungi and presence of aflatoxin is a major concern that received worldwide attention due to their deleterious effects on human and

animal health as well as their importance in international food trade [15]. Acute aflatoxicosis can also result in death; chronic aflatoxicosis on the other hand can result in cancer, immune suppression, and other “slow” pathological conditions [16]. Members of the genus *Aspergillus* also cause a large collection of diseases called Aspergillosis. Immunocompromised individuals are more prone to such infections than healthy persons. At present, the treatment of invasive aspergillosis is a challenge due to the diagnostic difficulty, the severity of the clinical conditions of the patients, and the limited number of antifungal drugs available [17]. Although, with the discovery of amphotericin B, there has been much progress in the treatment, there are still critical needs for new antifungal agents for treatment of diseases caused by aflatoxin [18-24].

Simarouba is one of the important medicinal plants with wide use. The bark and leaf extract of *S. glauca* is well known for its different types of pharmacological properties such as haemostatic, antihelminthic, antiparasitic, antidiarrheal, antipyretic and anticancerous [25]. The leaf, fruit, pulp and seed of *S. Glauca* are known to possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge [26]. Studies also revealed that it has strong inhibitory activity against protozoa [27]. In addition, Cuban folk medicine information also showed several other medicinal uses for this plant, including antihelminthic, antidiarrheal and aspergillosis action [28]. However, till date not many reports are available about its use as antifungal properties. Thus, the present study was carried out to analyse the antifungal properties of this broadly used medicinal plant against two human pathogens *Fusarium oxysporum* and *Aspergillus parasiticus*, for which no satisfactory drugs are still not available.

2. Materials and Methods

2.1 Collection of plant Material: The fresh leaves of *Simarouba glauca* were collected from the College University campus. Healthy and

disease free plants were selected for obtaining the plant materials.

2.2 Processing of sample plants

The flesh and healthy leaves were properly washed with tap water and rinsed with distilled water. The rinsed leaves were then dried in an oven at a temperature of 35-40 °C for 3 days. The dried leaves were pulverized using a sterile electric blender to obtain a powdered form. The powdered form was stored in airtight glass containers, protected from sunlight until required for analysis. On the other hand, the fresh leaves were also properly washed with tap water and rinsed with distilled water. These washed fresh leaves were immediately used for obtaining methanolic and ethanolic fresh extracts.

2.3 Preparation of solvent extracts of plant samples

5 g of the air dried powdered leaves were taken in 50 ml methanol, and kept under gentle and continuous shaking on an orbital shaker (Stuart Scientific Orbital Shaker, UK) for 6 hours at 55 °C. The suspension so obtained was filtered using Whatman No. 1 paper to obtain the methanolic extracts of dried leaves (MD). The procedure was repeated twice to ensure exhaustive extraction of the plant material. On the other hand, the washed fresh leaves were pulverized with 50 ml methanol using sterile electric blender, and the suspension obtained was then filtered using Whatman No. 1 paper to obtain the methanolic extracts of fresh leaves (MF). The same procedures were followed by using ethanol 99.9% to obtain ethanolic extracts of dried leaves (ED) and ethanolic extracts of fresh leaves (EF).

2.4 Test fungal strain

The fungal strains *Fusarium oxysporum* and

Aspergillus parasiticus were obtained from Microbiologia Laboratories.

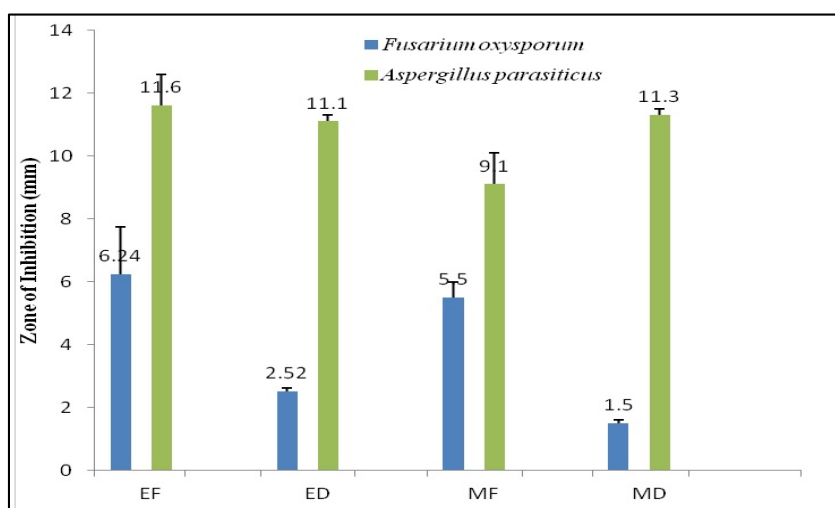
2.5 Antifungal assay: Antifungal activity was studied by agar well diffusion method [29]. The Potato Dextrose Agar (PDA) medium was poured into sterile petriplates and allowed to solidify. The fungal inoculum was seeded on PDA medium. Then wells (5 mm in diameter) were made in the medium using sterile cork borer. 200 µl each of the different extracts were transferred into separate wells. The plates were incubated at 27 °C for 72 hrs. After incubation they were observed for the presence of clear zones of inhibition around the well indicating antifungal activity. For each treatment three replicates were maintained and the zones of inhibition were measured in millimetres (mm).

3. Results

The antifungal assay showed that *Simarouba glauca* has antifungal property against the tested fungi *Fusarium oxysporum* and *Aspergillus parasiticus*. In this analysis, ethanolic extracts of fresh leaves (EF) were found to be more effective as compared to the methanolic extracts of the fresh leaves (MF) against the growth of the fungi (Table 1 and Graph 1). Also, the methanolic extracts of the fresh leaves (MF) were found to be more effective than methanolic extracts of dried leaves (MD) against *Fusarium oxysporum*. However, in case of *Aspergillus parasiticus*, the methanolic extracts of dried leaves (MD) were found to be comparatively more effective in comparisons to the methanolic extracts of fresh leaves (MF). As a whole, the present study showed that *S. glauca* has antifungal property against the tested fungi. These findings also validate the use of this plant for its antimicrobial property. The results also showed that *S. glauca* is more effective against *Aspergillus parasiticus* as compared to *Fusarium oxysporum*.

Table 1: Observation table showing different leaf extracts and the corresponding zones of inhibition (in millimetres) against *Fusarium oxysporum* and *Aspergillus parasiticus*.

S. No	Leaves Extract	Zone of Inhibition (mm)	
		Fungal Species	
		<i>Fusarium oxysporum</i>	<i>Aspergillus parasiticus</i>
1.	Ethanolic extracts of fresh leaves (EF)	6.24±1.50	11.60±1.0
2.	Ethanolic extracts of dried leaves (ED)	2.52±0.20	11.10±0.20
3.	Methanolic extracts of fresh leaves (MF)	5.50±0.50	9.10±1.0
4.	Methanolic extracts of dried leaves (MD)	1.50±0.10	11.30±0.20

**Graph 1:** Comparative zones of inhibition of different solvent extracts against *Fusarium oxysporum* and *Aspergillus parasiticus*

4. Discussion

From the present analysis, it can be drawn that *Simarouba glauca* has antifungal properties against the tested fungi. The extracts of this plant were however found to be more effective against *Aspergillus parasiticus* as compare to *Fusarium oxysporum* (Table 1 and Graph 1). Fungal diseases caused by *A. parasiticus*, *F. oxysporum* and other fungi once established in human host are often difficult to treat [30, 31] and the present antifungal agents commonly use have toxicity problems on the hosts organism as well, as both the pathogen and the hosts have eukaryotic set up of cellular organisation. In addition to these already complicated conditions in treatment of fungal diseases in human, the lack of accurate diagnostic techniques further limits the

effectiveness in treatments of the existing fungal diseases. Fungi such as *Fusarium oxysporum* f. sp. *lycopersici*, which causes vascular wilt in plants and *Aspergillus parasiticus*, an important aflatoxin producing fungus are of great threats to immunocompromised humans [32, 33]. Till now, approximately 15 species of *Fusarium* have been reported to cause human and animal diseases. The common species includes *F. solani* (commonest), *F. oxysporum*, *F. verticoides*, *F. proliferatum* and *F. anthophilum*. Four species of *Fusarium* namely *F. solani*, *F. moniliforme* (*F. verticilloides*) and *F. oxysporum* are accounted for more than 95% of infections in human [34, 35]. *Fusarium* spp. can cause superficial and subcutaneous infections, such as onychomycosis and keratomycosis, in humans [36]. Such

infections are rare and tend to respond well to antifungal agents. In contrast, infections caused by *Fusarium* spp. such as *F. proliferatum* can result in disseminated fusariosis in immunocompromised hosts such as hematopoietic stem cell transplantation (HSCT) recipients and patients with severe and prolonged neutropenia. Such infections are mostly fatal with virtually a 100% death rate for persistently neutropenic patients with disseminated disease.^[37] Some data reported that fungi such as *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp. or other fungi such as *Fusarium* spp. can also lead to cancer^[38, 39]. In India, a well-known outbreak involving *Fusarium* species among humans was the scabby grain intoxication reported from Kashmir^[40].

Aspergillus flavus and *Aspergillus parasiticus* produce the most potent naturally present carcinogenic mycotoxin aflatoxins, which contaminate various foods and feeds^[41]. Aflatoxin contaminations are often unavoidable process due to ubiquitous nature of the fungi producing them and production of abundant spores that disperse into the environment by air^[42]. Thus, foods contaminated with these toxigenic fungi and presence of aflatoxin is a major concern, which has received worldwide attention due to their deleterious effects on human and animal health as well as their importance in international food trade^[15]. Recently, there has been much resurgence and revival of interest in indigenous systems of medicines and traditional herbal remedies, which are regarded as less toxic with minimal or no side effects, cost effective, readily available and easily affordable^[43-46]. Interest in herbal drugs is growing due to their low toxicity, efficiency and absence of side effects^[47]. Thus, analysis and screening of medicinal plants documented in various reports are one way of the necessity approaches in encountering the ever increasing fungal diseases in human, where most fungi have become tolerant or less effective to most of the

currently available pharmaceutical agents. The present forms of analysis of the efficacy of the rich medicinal knowledge reported in traditional systems of medicine can contribute significantly in achieving important alternative solution to the current drug tolerance properties prevalent in fungal disease treatments.

5. Conclusions

The current findings can be used in further exploration of this medicinal plant in isolation of the antifungal agents responsible for these properties. In addition, the purified extracts of this plant can be explored for its use in preserving household foods and grains from contamination by mycotoxin producing fungi. As reports suggested that most of the available antifungal therapies are generally of limited value due to toxicity problems, the antifungal agents present in this plant can be isolated, tested and compared with the currently available drugs. Since aflatoxin contaminations have become an unavoidable process due to ubiquitous nature of the fungi producing them and their production of abundant spores that disperse into the environment by air, the currently reported medicinal plant and other potential antifungal plants can be useful in antimicrobial food packaging so that food items are less contaminated by fungi and fungal spores. Also, incorporation of the plant extracts in grains and food packaging can help in increasing the shelf-life of these products and preventing frequent exposure to mycotoxins.

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7. Conflict of Interest

There is no conflict of interests amongst the authors while conducting the analysis. The authors work cooperatively in bringing out the present analysis to its best form.

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