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RESEARCH ARTICLE

IN VITRO ANTIFUNGAL ACTIVITIES OF *SIMAROUBA GLAUCA* AGAINST *ASPERGILLUS PARASICTUS*

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ABSTRACT

Since time immemorial *Simarouba glauca* have been used as a natural medicine in the tropics. *Aspergillus flavus*, *A. parasiticus*, and *Penicillium puberulum* are well known transmitters of aflatoxin. Crude methanol and ethanol extracts from fresh and dried leaves of *Simarouba glauca* were tested for their inhibitory activity against pathogenic aflatoxin producing fungus *Aspergillus parasiticus.* Screening for the antifungal activity using well diffusion assay showed the inhibition against the tested fungi. Ethanolic extracts were found to be more effective as compared to methanolic extracts against the fungus. The present study shows that *Simarouba glauca* could be new a source for antifungal agent. The continuance of this study should include the isolation of the compounds responsible for the antifungal activity present in this medicinal plant.

INTRODUCTION

A large number of higher plants have been used as a source of drugs by mankind for several thousand years. About 35,000 to 70,000 plant species have been used for production of herbal medicine. Husain (1991). In the past few decades, a worldwide increase in the incidence of fungal infections has been observed**.** Among the Asian countries India and China are known to have large number of medicinal plants. Raven (1998). Approximately 7500 species out of 17,000 plants are known as medicinal plants in India. Shiva (1996). Medicinal plants can be used as splendid source of antimicrobial agents for development of many valuable and powerful drugs. Srivastava *et al*. (1996). However, only few hundred plant species have been examined for antibacterial characteristics but still a large number of medicinal plants have not been tested. Balandrin *et al*. (1985). So screening of various natural compounds such as crude extracts, chromatographic fractions or purified compounds is the need of today's world for development of an effective drug and it should also be simple, rapid, efficient, trustworthy, sensitive, safe and cost-effective. Satyajit *et al*. (2007). With an increase in the antibiotic-resistant strains of microorganisms, traditional plants are being investigated for their antibacterial and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties. Sharma *et al*. (2010) Autopsy data indicated that more than half of the patients who die with malignancies are infected with fungi such as *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp. or other fungi such as *Fusarium* spp. Andriole (1998); Walsh (1992) With the development of resistance in known fungal pathogens and the emergence of fungal pathogens intrinsically resistant to the currently available antibiotics, it is important that novel antifungal agents be identified and developed. Ficker (2003). Although, the discovery of amphotericin B, there has been much progress in this field, there is still a critical need for new antifungal agents to treat life threatening invasive mycoses. Andriole (1998); Groll *et al.* (1998); Georgopapadakou and Walsh (1996); Vazquez *et al.* (1993). Intravenous amphotericin B has been the mainstay of effective therapy for invasive fungal infections. However, almost every patient who that are treated with this drug develops some abnormality in renal function. Andriole (1998); Bodey (1993); Andriole (1962). The present study was carried out to assess the effect of methanolic and ethanolic extracts of *Simarouba glauca* against potent carcinogenic aflatoxin producing fungal parasite *Aspergillus parasiticus.*

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MATERIALS AND METHODS

Collection of plant Material

Simarouba glauca is a medium sized evergreen tree (height 7-15 meters) with tap root system and cylindrical stem. The leaves of the plant were collected from the College University

campus. Only disease free healthy plants were collected for this study.

Processing of sample plants

The leaves of the plant were properly washed with tap water and rinsed with distilled water. The rinsed leaves were dried in an oven at a temperature of 35-40^{o}C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of these plants is 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.

Preparation of solvent extracts of plant samples

5g of the air dried and 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^{o}C. The suspension was then filtered using Whatman No. 1 paper to obtain the methanolic dried extract. The procedure was repeated twice to ensure exhaustive extraction of the plant material. On the other hand, the washed fresh leaves were pulverized with 50ml methanol using sterile electric blender, and the suspension was then filtered using Whatman No. 1 paper to obtain the methanolic fresh extract. These same procedures were also performed by using ethanol 99.9% to obtain ethanolic dried and ethanolic fresh extracts.

Test fungal strain

The fungal strain *Aspergllus parasiictus* was obtained from *microbiologia* laboratories.

Antifungal assay

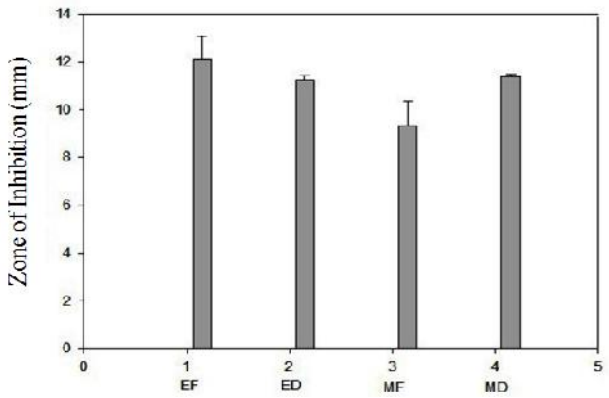
Antifungal activity was screened by agar well diffusion method (Andriole and Bodey 1994). The PDA medium was poured in to the sterile petriplate and allowed to solidify. The fungal inoculum was seeded on PDA medium. Then wells (5 mm) were made in the medium using sterile cork borer. 200µl of each of the 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 inhibition zone around the well indicating antifungal activity. For each treatment three replicates were maintained and the zone of inhibition was measured in millimetres.

RESULTS

The antifungal assay showed that *Simarouba glauca* has antifungal property against the tested fungus *Aspergillus parasiticus*. Ethanolic extracts of the fresh plant leaves were found to be more effective as compared to methanolic extracts against the growth of the fungus. On the contrary, the methanolic extracts of the air dried plant leaves were found to have slightly better antifungal effect than the ethanolic extracts of the air dried plant leaves. The detail observations are depicted in Table 1 and Graph 1. The present study showed that *Simarouba glauca* could be new a source for antifungal agent against *Aspergillus parasiticus*, which is responsible for contamination of grains, nuts and other storage foods by producing aflatoxin, a highly potent carcinogenic mycotoxin.

Table 1. Observation table showing different leaf extracts and the corresponding Zone of Inhibitions in millimetres

Sl.No	Leaf Extracts	Zone of Inhibition (mm)
1.	Ethanolic extracts of fresh leaves (EF)	12.10±1.00
2.	Ethanolic extracts of dried leaves (ED)	11.25±0.20
3.	Methanolic extracts of fresh leaves (MF)	9.35 ±1.00
4.	Methanolic extracts of dried leaves (MD)	11.40±0.10



Graph 1: Comparison of effects of different solvent extracts and their Zones of Inhibition

EF = Ethanolic extracts of fresh leaves, ED = Ethanolic extracts of dried leaves.
MF = Methanolic extracts of fresh leaves, MD = Methanolic extracts of dried leaves.

DISCUSSION

The present analysis on the antifungal activity of *Simarouba glauca* against *Aspergillus parasiticus* could be the first report on the study of this plant against the tested pathogenic fungus. Over the past 25 years, there has been a resurgence of worldwide scientific research in the fields of ethno pharmacology. The Western world has acknowledged the continued use of traditional medicines by the majority of third world countries, and the need for novel drug development. Hence, much of the pharmaceutical research in recent years has focused on the ethnobotanical approach to drug discovery. Light (2005). *A. parasiticus* is one of the normal soil-borne fungi growing on both living and decaying organic matter. It is also one of the aflatoxin producing fungi, the other genus being *Aspergillus flavus*. Many common household foods contaminated by *A. parasiticus*, can be unhealthy for already immunocompromised individuals. Aflatoxicosis, a type of food poisoning due to ingestion of such 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, which has received worldwide attention due to their deleterious effects on human and animal health as well as their importance in international food trade. Mishra (2003). The types of diseases caused by *Aspergillus* are varied, ranging from an "allergy"-type illness to various life-threatening such as Aspergillosis. Aspergillosis is a large collection of diseases caused by members of the genus *Aspergillus*. Aflatoxins are known to be potent carcinogens and hepatotoxic agents and pose a severe hazard to animal and human health. Stoloff (1977). These mycotoxins are also

associated with both toxicity and carcinogenicity in human and animal populations. Eaton (1994); Newberne (1969); Peers (1973); Sansing (1976). In classical epidemiology, several studies have linked liver cancer incidence to estimated aflatoxin consumption in the diet. Peers and Linsell (1973); Li *et al.* (2000). The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis can result in death; chronic aflatoxicosis can result in cancer, immune suppression, and other "slow" pathological conditions. Hsieh (1998). Moreover, aflatoxin contaminations are mostly unavoidable because the fungi producing them are ubiquitous and sporulates abundantly, thereby dispersing spores into the environment by air. Thus, because of their ubiquitous presence, people are probably constantly exposed to *Aspergillus* spores. Exposure to aflatoxins in the diet is considered an important risk factor for the development of primary hepatocellular carcinoma, particularly in individuals already exposed to hepatitis B. 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. Verweij *et al.* (2007). As aflatoxin contamination is an unavoidable event, the identification of plants such as *S. glauca* could prove useful in antimicrobial food packaging. Also, incorporation of the plant extracts in food packaging can help in increasing the shelf-life of food products and preventing frequent exposure to aflatoxins. The present analysis thus justified the justify future researcher for isolation of effective antifungal agent that can be used as an alternatives to the present problems of fungal diseases.

Conclusion

From the above studies it can be concluded that *Simaroubou glauca* may be new source for antibiotics with suitable bioactive compounds. The antifungal activity of this plant can be explored for treatment of various diseases against human pathogens, since other earlier reports showed the property of this plant as antibacterial and antifungal agent. The continuance in this study can lead to the isolation of the compounds responsible for the antifungal activity present in *Simaroubou glauca*.

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Conflict of interest

There is no conflict of interests amongst the authors while conducting and compilation of the review. The authors have share equal contribution to bring out the review in its best form.

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