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Research Article

Antifungal and Phytochemical Screening of *Hyptis suaveolens* (L.Poit) Lamiaceae On Aflatoxin Producing Fungi

P. N. Rajarajan[1*], K. M. Rajasekaran[2], N. K. Asha Devi [3]
[1,2]Centre for Botanical Research, The Madurai College, Madurai-62011, India
[3]Department of Zoology, Thiagarajar College, Madurai-625009, India

***Corresponding author**
P. N. Rajarajan
Email: r.raja56@gmail.com

Abstract: The present communication deals with the antifungal and preliminary phytochemical screening on the *Hyptis suaveolens* (L. Poit) Lamiaceae. The plant is stimulant, carminative, antispasmodic antirheumatic, antisuporitic bath. It is also used for parasitical cutaneous diseases, infection of uterus, and as sudorific in catarrhal condition, headache, stomach, snuff to stop bleeding of the nose. The antimicrobial effect of *Hyptis suaveolens* leaves extract was evaluated methanol extracts was carried out by using fungus like, *Aspergillus flavus* and *Aspergillus parasiticus*. The in vitro antimicrobial evaluation was carried out by agar disc-diffusion method. Preliminary phytochemical screening shows the presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides etc. *Hyptis suaveolens* leaves may be considered as an alternative and effective inhibitor of *Aspergillus flavus* and *Aspergillus parasiticus*, in addition to offer some protective effect to the production of aflatoxins.
Keywords: *Hyptis suaveolens*, Medicinal plant, solvent extract, phytochemicals, aflatoxin

INTRODUCTION

Aflatoxin is the name for a group of toxins known as B1, B2, G1, G2, M1 and M2 (carcinogenic compounds) that are produced mainly by two fungi called *Aspergillus flavus* and *Aspergillus parasiticus* [1]. Aflatoxin B_1 is considered the most toxic and is produced by both *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin G_1 and G_2 are produced exclusively by *A. parasiticus* [2]. Aflatoxins have detrimental effects, so a number of Strategies have been developed to help prevent the growth of aflatoxigenic fungi as well as to decontaminate or detoxify aflatoxin contaminated foods and animal feeds.

The prevention of aflatoxin production includes all phases of food and feed production, because the mould contamination may occur in the field, during storage, as well as in transport [3]. Adequate storage with optimal temperature and humidity of grains and relative humidity and the hygiene in silos may decrease the growth of toxicogenic moulds [4]. The fungitoxic activities have been reported by *Hyptis suaveolens* which showed complete reduction in aflatoxins synthesis [5].

Hyptis suaveolens (L.) Poit. belongs to the *Lamiaceae* family and is widely used in folk medicine in various countries Phytochemistry essentially deals with the enormous different types of organic substances that are not only elaborate but also accumulated by plants. Natural's product is a source of synthetic and traditional herbal medicine and is still the primary health care system [6]. The plant *Hyptis suaveolens* (L.) poit commonly known as wilayati tulsi belonging to the family Lamiaceae and is an ethnobotanically important medicinal plant. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics almost all parts of this plant are being used in traditional medicine to treat various diseases [7, 8].

MATERIALS AND METHODS

Collection of Plant Material

The plant was obtained from the Madurai region, Tamilnadu (Fig. 1). This was identified and authenticated at Centre for Botanical Research, The Madura College Madurai.

Fig. 1: *Hyptis suaveolens* (L.) Poit.

Preparation of Extract

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of the methanol solvent and distilled water [9] (Fig. 2). The extracts obtained were filtered through Whatman filter paper No. 1 and were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish brown substances were obtained and stored in refrigerator for prior to use (Fig. 3).

Fig. 2: Preparation of Extract

Fig. 3: Leaf extract

Fig. 4: Fungal Growth on Czapex broth Medium

Experimental Micro organisms

Aflatoxin producing toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* were used in this study to show the antifungal activity against the plant extracts.

Procedure for Performing the Disc Diffusion test

The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. And they were allowed to cool under laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution. The readily prepared sterile discs were loaded. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) (Table 1).

Histochemical Analysis

Transections of leaf were taken by free hand. Histochemical tests were performed on fresh plant materials according to the methods of Johansen and Guerin [10, 11]. The positive tests were noted as present (+) and absent (-).

RESULTS AND DISCUSSION

Plants are known to have beneficial the therapeutic effect documented in traditional Indian system of medicine. Much work has been done on ethno medicinal plant in India. Interest in a large number of traditional natural products has increased. The results obtained for the antimicrobial tests of *Hyptis suaveolens* are presented in (Table 1). The methanol extract effectively inhibited all the test organisms. The methanol extract was chosen for further studies because of its high inhibitory activity compared to ethyl acetate and hexane extract. Growth inhibition assays were used in this study to determine the microbial growth inhibition by crude extract from *Hyptis suaveolens.*

The result of the present study indicate that methanol extract of *Hyptis suaveolens* leaf has good antimicrobial activity, while that of hexane and ethyl acetate extract have less antimicrobial activity This was showed by measuring the zone of inhibition in well diffusion method. As methanol extract showed good zone of inhibition, the further studies was concentrated on this extract.

In the present antifungal activity of plant extract towards aflatoxic fungi are reported and it was observed that active constituent of plant material seep out in organic solvent to display biological activity. The adverse effects of chemical pesticides on environment and human health are burning issues. Hence some extracts of ethanomedically important higher plant

species were tested for their antifungal activity. Such plant products would be biodegradable and safe to human health. Further Phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound. Histological results indicate presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides in leaves (Table 2).

Table 1: Antifungal activity of methonolic extract of *Hyptis suaveolens*

Sl. No.	Test Microorganism	Zone of inhibition (mm) in different concentration				
		Control	50 µg/ml	100 µg/ml	150µg/ml	200µg/ml
1	*Aspergillus flavus*	C	18	22	25	28
2	*Aspergillus parasiticus*	C	16	19	21	24

Table 2: Histochemical Test of *Hyptis suaveolens*

Sl. No.	Test	Leaf
1.	Volatile oil	+
2.	Starch	+
3.	Protein	+
4.	Tannin	+
5.	Saponin	+
6.	Fat	+
7.	Alkaloids	+
8.	Glycoside	+

CONCLUSION

According to the present results, *Hyptis suaveolens* leaves revealed a fast and steady anti-*Aspergillus flavus and Aspergillus parasiticus* property with a strong inhibition of the mycelial growth, fungi spore germination and aflatoxins production. Based on this study, it can be concluded that the *Hyptis suaveolens* leaves, if applied in sufficient amounts, possesses fungitoxic activities, inhibiting the growth of *Aspergillus flavus*, and *Aspergillus parasiticus* and thus could be considered as an alternative inhibitor of the survival of this fungi in foodstuffs, in addition to offer some protective effect to the production of aflatoxins.

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