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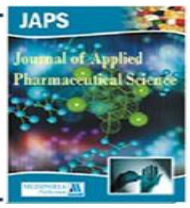

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In-vitro Antifungal activity of *Sapium sebiferum* L. against *Aspergillus niger* and Aflatoxigenic *Aspergillus flavus*

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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 methanolic, ethanolic, chloroformic and petroleum ether extracts of *Sapium sebiferum* leaves were investigated for their antifungal activity against *Aspergillus niger* and Aflatoxigenic *Aspergillus flavus*. Results obtained showed that all the extracts reduced colony growth by 1-32%. It was found that among all the leaf extracts, methanolic and ethanolic extracts have maximum percentage growth inhibition (26%) against *Aspergillus flavus*, while methanolic extracts showed maximum percentage growth inhibition (32%) against *Aspergillus niger*.

Keywords: Antifungal activity, *Sapium sebiferum*, Aflatoxigenic *Aspergillus flavus*, *Aspergillus niger*.

INTRODUCTION

Fungi are increasingly important causes of acute or chronic deep-seated human infections, especially recurrent mucosal, cutaneous or nail infections that may be severe in debilitated or immunocompromised individuals. The small number of drugs available for their treatment, most of them fungi static and emerging resistance permanently encourage the search for alternatives and led us to find them among low cost and low toxicity traditional therapies and natural products (Cavaleiro *et al*., 2006). *S. sebiferum* is commonly known as Chinese tallow tree. It is a tree in the spruge family *(Euphorbiaceae)*. At maturity, it typically reaches a maximum height of 15m. Its bark is reddish-brown with wide fissures and narrow strips. The branches are typically long and dropping. The twigs are slender and waxy. The leaves are alternate and deciduous, broad rhombic to ovate, 3-8cm wide and have a smooth margin. *S. sebiferum* is monoecious. The flowers are greenish-yellow in terminal spike like inflorescence up to 20 cm long fruits are three lobed, three valved capsules about 1-2 cm long and 2 cm long. As the capsule mature, their colour changes from green to nearly black. The capsule walls fall away and expose three globose seeds with a white, tallow-containing covering (Godfrey, 1988). *S. sebiferum* is native to China and Japan and has been introduced into most of the subtropics. The outer covering of the seeds contains a solid fat known as Chinese vegetable tallow and the kernels produce a drying oil called stillingia oil. Candles, soap, cloth dressing and fuel were made from the tallow. The oil is used in machine oils, as a crude lamp oil, in making varnishes and paints. The oil is also report used in Chinese medicine as an emetic or purgative. A black dye can be made by boiling leaves of *S. sebiferum* in alum water (Duke and Ayensu, 1985).

MATERIALS AND METHODS

Collection of plant material

The leaves of *Sapium sebiferum* was collected from Raja Ji National Century, Dehradun (Uttarakhand). The plant was well identified by Dr. Prashant, Botanical Survey of India, Dehradun. The leaves are shade dried and powdered using mortar pestle.

Extraction of Plant Material

100 gm of air dried powdered leaves were extracted with different solvent i.e. methanol, ethanol, chloroform and petroleum ether. After extraction process was completed filtrate, which was obtained by the extraction, were concentrated in Rotary Evaporator (Butchi Type) till all the solvent evaporates. Before putting the antimicrobial activity, all plant extracts i.e. methanol, ethanol, petroleum ether and chloroform was stored at 4^oC.

Fungal Growth inhibition Assay

Antifungal activity of plant extracts against *A. niger* and *A. flavus* was determined by fungal growth inhibition assay as described by Fiori *et al.*,(2000) with some modification. The filtered sterilized leaf extract was mixed with molten Potato dextrose medium (PDA) to provide desired concentration. An 8mm diameter disc or 18×10^4 spore/ml in preformed well (8 mm) were added into the presterilised PDA medium and incubated at 28 $\pm2^oC$. For the control treatment only PDA medium was used without plant extract. The colony diameter was measured after 72 hrs and inhibition percentage of the fungal growth in relation to control treatment was calculated according to the given formula:

$$I = C - T/ C \text{ X } 100$$

Where I = percentage inhibition
C = radial growth in control
T = radial growth in treatment (Test)

RESULTS AND DISCUSSION

Several authors (Morozumi., 1978, Azzour and Bullerman.1982, Bahk and Marth., 1983, Yin and Cheng., 1998) 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 (Yin and Cheng, 1998, Greyer and Harborne,1994). Different fractions of *S. sebiferum* leaves investigated and were found to inhibit the growth of Aflatoxigenic *A. flavus* and *A. niger* (Table1-8). Different concentrations i.e. 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm of methanolic, ethanolic, chloroformic and petroleum ether extracts of *S. sebiferum* leaves were tested for their efficacy against *A. niger* and *A. flavus* (Aflatoxin producing). All the extracts were found to inhibit the growth of selected fungi. The percentage growth inhibition of *A. flavus* was found maximum with methanolic and ethanolic extract i.e. 26%, followed by chloroformic (24%) and petroleum ether extracts (22%) at 4000 ppm concentration. (Table 1-4). In case of *A. niger*, the methanolic extract had found maximum percentage growth inhibition (32%) followed by chloroformic (29%), petroleum ether (26%) and ethanolic (22%) at 4000 ppm concentration (Table 5-8). The aqueous extracts of *S. sebiferum* leaves had no antimicrobial effect against the any test fungi.

Table 1. Effect of methanolic extract of *S. sebiferum* leaves against *A. flavus.*

Concentration (ppm)	*Sapium sebiferum*(Leaves)	
	Radial diameter in mm after 72 hrs	Percentage inhibition (%)
Control	28.5±3.0	0
1000	25.5±1.5	10
2000	23.5±1.0	17
3000	22.5±0.0	21
4000	21.0±1.0	26

Table 2. Effect of ethanolic extract of *S. sebiferum* leaves against *A. flavus.*

Concentration (ppm)	*Sapium sebiferum*(Leaves)	
	Radial diameter in mm after 72 hrs	Percentage inhibition (%)
Control	28.5±3.0	0
1000	24.0±0.5	15
2000	23.0±1.5	19
3000	22.0±0.5	21
4000	21.5±0.5	26

Table 3. Effect of chloroformic extract of *S. sebiferum* leaves against *A. flavus.*

Concentration (ppm)	*Sapium sebiferum*(Leaves)	
	Radial diameter in mm after 72 hrs	Percentage inhibition (%)
Control	33.5±1.0	0
1000	32.5±1.0	3
2000	31.5±2.0	7
3000	27.0±3.0	19
4000	25.5±1.5	24

Table 4. Effect of petroleum ether extract of *S. sebiferum* leaves against *A. flavus.*

Concentration (ppm)	*Sapium sebiferum*(Leaves)	
	Radial diameter in mm after 72 hrs	Percentage inhibition (%)
Control	33.5±1.0	0
1000	32.0±0.5	4
2000	27.5±1.0	18
3000	27.5±1.5	18
4000	26.0±2.0	22

In conclusion, different solvent extracts of studied plant leaves showed antifungal activity. Among the extract tested, methanolic and ethanolic extract of leaves showed higher antifungal activity against *A. niger* and *A. flavus*. This gives an indication of the presence of promising antifungal compounds. Authors also designed further study of phytochemicals 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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