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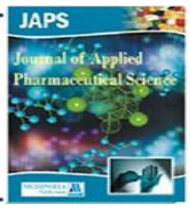

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Evaluation of *In-vitro* antibacterial and anticandidal activity of *Sapium sebiferum* L.

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ABSTRACT

The *Sapium sebiferum* leaf extracts were determined for their antimicrobial activities against *Staphylococcus aureus, Micrococcus luteus ,Pseudomonas aeruginosa, Salmonella typhi, Klabsiella oxytoca, Listeria monocytogenes, E.coli, Saccharomyeces cereviceae* and *Candida albicans*. The antimicrobial activities of these strains were compared with standard antibiotics (amoxicillin). Results obtained showed that all the extracts except aqueous (hot and cold) were effective against all the microbial strains. The methanolic extract of *Sapium sebiferum* leaves was most effective against all the bacterial strains and thus displayed highest zone of 21.0 mm at concentration of 100 mg/ml against *Listeria monocytogenes* (MIC value is 25 mg/ml). *Salmonella typhi* and *Klabsiella oxytoca* did not show any activity. The anticandidal activity of methanolic, ethanolic and petroleum extracts showed maximum inhibition zone of 24.0 mm (MIC value is 25mg/ml).

Keywords: Antibacterial, Anticandidal, *Sapium sebiferum*.

INTRODUCTION

Sapium 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 were 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^{o}C. The aqueous extracts were made by dissolving powered leaves in cold and hot water for 1 hour.

Micro-organism Collection and Maintenance

The micro-organisms used in this study- *Staphylococcus aureus MTCC902, Micrococcus luteus MTCC106 ,Pseudomonas aeruginosa MTCC424, Salmonella typhi MTCC733, Klabsiella oxytoca MTCC109, Listeria monocytogenes MTCC657, E.coli MTCC443, Saccharomyeces cereviceae MTCC2627* and *Candida albicans MTCC3017* were obtained from standard culture collection centre like MTCC Chandigarh. The organisms were stored on agar slant in Mccartney bottles and kept in the refrigerator, prior to subculture.

Antimicrobial Assay

The antimicrobial activity of different extracts were determined by agar well-diffusion method (Dahunukar and Garkel, 1995).The molten Mullar Hinton Agar was added to pre-sterilised plates and 0.1 ml of 12-16 hrs incubated culture of microbial strains (diluted according to Macfarlein constant) were spreaded over the agar plates. Wells were bored into the medium adding 0.1 ml of extracts (extracts were dissolved in ≤30% DMSO).The aqueous extracts were used as such. The plates were kept in a sterilized incubation chamber for 2 hrs to facilitate diffusion of the antimicrobial agent into the medium. The plates were then incubated at 37ºC for 24 hrs in BOD incubator and the diameter of the zone of inhibition were measured in millimeter. The efficacy of extracts against clinically important microbial strains was compared with standard antibiotic Amoxicillin. Strategies of comparison are given in the Table 1.

Table 1. Strategy of comparison for extracts.

Zone size	Interpretation
Equal to wider than or not more than 3 mm smaller than the control(Amoxicillin)	Susceptible
Zone size greater than 3 mm, but smaller than the control by more than 3 mm	Intermediate
Zone sizes 3 mm or less	Resistant

Minimum Inhibitory Concentration

Broth Dilution Assay: The different sets of the sterilized nutrient broth tubes were prepared for bacterial strains and sabouraud broth tubes for fungal strains .Each set having six screw cap tubes containing 25 µl of different concentration (mg/ml) viz.100, 50, 25, 12.5, 6.25 of the crude extracts respectively. The last tube was without extract considered to be positive control showing growth. 10 ml of the respective test organism were added in each tube. Tubes having bacterial test organism were incubated at 37ºC for 16-24 hrs, while tubes having fungal test organism were incubated at 28 ºC for 72 hrs. After the incubation period, observed the tubes for growth. The tubes were analyzed according to turbidity (growth of bacteria). The tubes in which the extract is present in high concentration great enough to inhibit microbial growth remain clear. MIC means the concentration of extracts present in the last "clear" tube. Same procedure was followed for each plant extract and each test organisms.

RESULTS

Results obtained showed that all the microbial strains have found sensitive to all these extracts except aqueous (cold and hot water) extract (Table 2).

Table 2. Antimicrobial activity of *Sapium sebiferum* L against different microbes.

Bacterial strains	MeOH	EthOH	$CHCL_3$	Pet Eth	Cold Water	Hot Water	Amoxicillin*
Staphylococcus aureus MTCC902	19.5 (50)	18.0 (50)	13.5 (50)	12.5 (50)	-	-	18.0
Micrococcus luteus MTCC106	16.5 (50)	16.0 (50)	15.0 (50)	13.5 (50)	-	-	10.0
Pseudomonas aeruginosa MTCC424	21.0 (25)	14.0 (50)	15.5 (50)	16.0 (50)	-	-	11.0
Salmonella typhimurium MTCC733	-	-	-	-	-	-	17.0
Klabsiella oxytoca MTCC109	-	-	-	-	-	-	12.0
Listeria monocytogenes MTCC657	23.0 (25)	16.0 (50)	17.0 (50)	15.5 (50)	-	-	12.0
E.coli MTCC443	19.0 (50)	18.0 (50)	10.5 (50)	12.0 (50)	-	-	18.0
Saccharomyeces cereviceae MTCC2627	20.0 (25)	18.5 (50)	10.5 (50)	12.0 (50)	-	-	20.0
Candida albicans MTCC3017	24.0 (25)	24.0 (25)	18.0 (50)	21.5 (25)	-	-	21.0

MeOH- Methanolic, EthOH- Ethanolic, $CHCL_3$-chloroformic , Pet Eth- Petroleum Ether, CW-cold water, HW- Hot Water extracts, *- standard antibiotic solution Amoxicillin.
*** MIC values are given in brackets (mg/ml).
** zone of inhibition in mm.

All the extracts showed good efficacy against *Micrococcus luteus, Pseudomonas aeruginosa , Listeria monocytogenes* and *Candida albicans* except *Staphylococcus aureus, E.coli and Saccharomyeces* (showed intermediate efficacy). None of any extract had efficacy observed against *Salmonella typhi* and *Klabsiella oxytoca.* (Figure 1,2,3,4.).

DISCUSSION

The activity of plant extracts against bacteria have been studied for years, but in a more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluation have been published based on the traditional use of Chinese, African and Asian plant based drugs (Suffredini et al., 2004). In the present study, all extracts of the leaves of *S.*

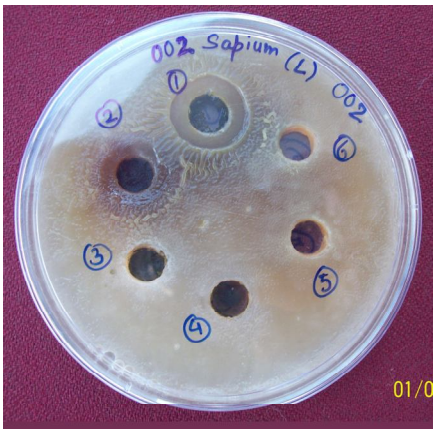

Fig 1. Antimicrobial activity of *Sapium sebiferum* leaves against *S. aureus*.

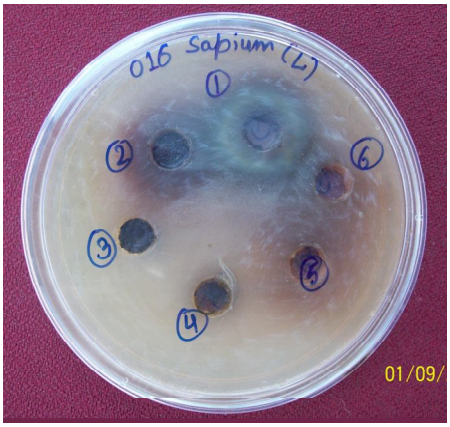

Fig 2. Antimicrobial activity of *Sapium sebiferum* leaves against *E. coli.*

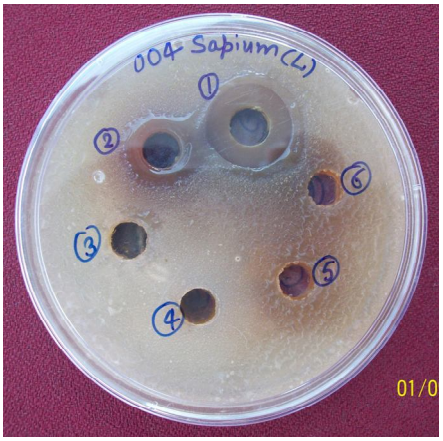

Fig 3. Antimicrobial activity of *Sapium sebiferum* leaves against *P. aeruginosa*.

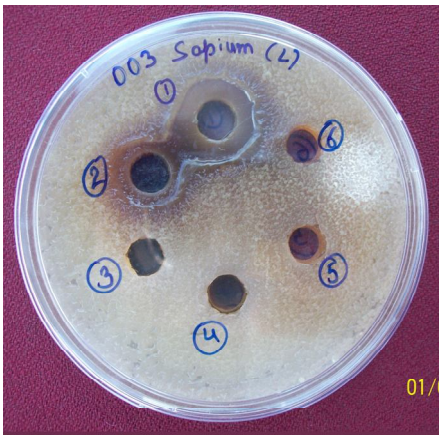

Fig 4. Antimicrobial activity of *Sapium sebiferum* leaves against *M. luteus.*

sebiferum inhibited the growth of all microbial strains, but their effectiveness varied. All the extracts showed higher activity against all the strains. The medicinal properties of the plant could be attributed to presence of one or more of the detected plant natural products. It should be noted that steroidal components are of importance and interest in pharmacy due to sex hormones (Okwu., 2001).The potential for developing antibacterial from higher plants appears rewarding as it will lead to the development of a phytomedicine, to act against microbes. In conclusion, *S.sebiferum* leaf extracts possess a broad spectrum of activity against a panel of microbial strains responsible for the most common bacterial diseases. These promissory extracts open the possibility of findings new clinically effective antimicrobial compounds.

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