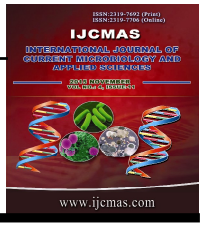
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 336-341

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 336-341**
http://www.ijcmas.com



Original Research Article

Study on Antibacterial and Antifungal Activities of *Sterculia lychnophora* Extracts

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Keywords

Sterculia lychnophora, Antibacterial and antifungal activity

A B S T R A C T

The study investigated the crude extracts obtained from the seeds of the plant *Sterculia lychnophora* for antibacterial activity and antifungal activity. The antimicrobial properties were determined by Agar cup method using *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Candida albicans, Klebsiella pneumoniae and Salmonella typhi* as test organisms. The diameter of zone is measured and recorded in mm. Results obtained in the present study revealed that the four extracts of plant *Sterculia lychnophora* posses potential antibacterial activity against *E. coli, S. aureus* and *S. typhi and* antifungal activity against *C. albicans*. Whereas *S. pyogenes* and *K. pneumoniae* were found to be resistant against all extracts under study.

Introduction

Natural products are believed to be important source of new chemical substances which have potential therapeutic effects. Medicinal plants are extensively investigated both *in vitro* and *in vivo* to examine for their potential activities (Pancharee, 2010). The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents that lead to repeated use of antibiotics and insufficient control of the disease (Prashanth Kumar *et al.*, 2006). Many secondary metabolites of plant

Chemicals are derived biosynthetically from plant primary metabolites. The secondary metabolites can be classified into several groups on the basis of their chemical classes (Angela *et al.*, 2008). Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc (Prashanth Kumar *et al.*, 2006).

Sterculia species have been extensively used in traditional medicine in the various countries where they are available. In the Solomon Islands, *S. lepidoto- stellata* is used to treat common cold. *S. macrophilla* is used

as an aphrodisiac in Java. In Australia, *S. quadrifolia* is used as peanut tree. In Guiana, *S. rubigenosa* used as fruit diet. *S. scaphigera*, in Thailand, is used to treat fever (Angela *et al.,* 2008) and also shows the analgesic, anti-inflammatory, antioxidant and antiulcer activity (Dhage *et al.,* 2013). *S. shillinglawii* is also said to treat fever and action is tonic (Angela *et al.,* 2008). *S. tragacantha* is used in the treatment of boils, diarrhea, dyspepsia, fever, gonorrhea, snake bite, syphilis, tapeworm (Irvin, 1961), used to treat malaria (Denis *et al.,* 2002) and Antibacterial activity (Orisakeye and Olugbade, 2012). *S. quinqueloba* shows anti-mycobacterial activity *in vitro* (Wilson *et al.,* 2015).

For a long history Chinese people used the dried and ripe seed of *Sterculia lychnophora* is a well-known Chinese medicine. In Cambodia it is used as tea. This traditional drug is known for its remedy and prevention against pharyngitis. Since ancient times in China it has also been used for the treatment of tussis and constipation (Coles, 1981) and in India used to treat heavy menopausal bleeding (Sharma *et al.,* 2012). The presence of flavonoids, terpenoids, phenolics, polysaccharides and histamines in other species of genus *Sterculia* were reported (Ru-Feng Wanga *et al.,* 2003). Cerebrosides compounds showed neuroprotective effect which is present in *Sterculia lychnophora* (Ru-Feng Wang *et al.,* 2013). In *Sterculia lychnophora* species showed secondary metabolites like tannins, saponins, flavonoids, phenols, terpenoids, alkaloids and lipids (Palve Anuradha *et al.,* 2015). The *Sterculia lychnophora seed* has been claimed to treat cough, pain and clear phlegm and used as antipyretics (Pancharee, 2010). *In–silico* docking study showed that ligand 1 (soyacerebrosids 1) and ligand 2 (1-O –beta-D- glucopyranosyl-(2S,3R,4E,8Z)-2-((2-hydroxyoctadecanoyl) amido)-4,8-octadecadiene-1,3-diol) was having high E total score against 3IFN protein but better result was shown by the ligand 1 since it was having highest negative score compared to ligand 2 (Shetty *et al.,* 2014). Thus the present study was aimed to examine the *Sterculia lychnophora* extract to show antibacterial and antifungal activity.

Materials and Methods

Plant material collection

The plant material Malva nut (*Sterculia lychnophora)* was bought from APMC market, Vashi, Navi Mumbai in May 2013. The plant was authenticated by Dr. Shankaran Potty comparing herbarium specimen at Department of Botany, Sathaye College, Mumbai. The damage less seeds were dried in hot air oven at 60^{o}C-65^{o}C for 12hrs and blended into powder, this powder of *Sterculia lychnophora* were used for aqueous and methanol extraction and phytochemical analysis.

Extraction

Extraction method used:

a. Hot Extraction (Soxhlet)
b. Cold Extraction

Hot extraction

The powder of *Sterculia lychnophora* which is ground using blender is used for the preparation of hot extracts using soxhlet apparatus. The condenser is supported by rubber-padded clamps attached to a 4 inch iron rod. Then condenser fits to thimble chamber. The thimble was prepared using Whatman's filter paper no.1 where 20g *Sterculia lychnophora* (Malva nut) powder is placed into it. Then these thimbles were inserted into extraction tube of soxhlet apparatus which are connected other extraction tube with tube. The round bottom

flask with solvent is boiled using heating mantle and maintained the temperature at 100^{o}C for water and 65^{o}C for methanol. This process is carried out until the color of solvent overflowing from thimble chamber turns to colorless. Later the extract was allowed to cool and then evaporated on water bath to reduce the volume 20ml which is stored in borosil bottle and refrigerated at to -4^{o}C. The hot extract used for antimicrobial assay and HPTLC analysis (Pimpliskar, 2004).

Cold extraction

For cold extraction 10g of seeds (*Sterculia lychnophora*) were weighed and crushed in mortar and pestle by adding 8ml of solvent (distilled water and methanol) and kept for 45mins. Then the extract was filtered using Whatman's filter paper no.1 and in similar way again 6-8ml of solvent added and filtered. At the end volume was made to 20ml by adding solvent and stored in an air tight bottle and then refrigerated at -4^{o}C and then this extract was then used for phytochemical tests and antimicrobial assay (Pimpliskar, 2004).

Antimicrobial activity

Preparation of cotton swabs

A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in culture tubes in the autoclave.

Experimental procedure

The sterilized nutrient medium (20 ml) was poured in sterilized Petri dishes under aseptic condition and allowed to solidify on a plane surface.

All the extracts were first brought to room temperature and then used for bioassay test against the microorganisms by Agar cup method. The sterile agar plates were inoculated with test cultures (Table 1) of *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Candida albicans, Klebsiella pneumoniae and Salmonella typhi* by dipping a sterile swab into inoculums. Excess inoculums were removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60^{o} after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed. After drying four wells were made with the help of flame sterilized cork borer of 8mm diameter made of stainless steel. Four wells were then loaded with 80µl of four extracts with the help of micropipette and were kept for diffusion in refrigerator at 4^{0}C for 45mins to 1hr. The plates were then placed for incubation at required temperature i.e. *Escherichia coli, Streptococcus pyogenes, and Salmonella typhi* was kept at 37^{0}C while *Staphylococcus aureus, Klebsiella pneumoniae and Candida albicans* at room temperature for 24hrs to 48hrs. The diameter of zone is measured and recorded in mm (Thamaraiselvi *et al.*, 2012).

Results and Discussion

The antibacterial activity of *Sterculia lychnophora* against test organisms is shown in table 2. Results obtained in the present study revealed that the four extracts of plant *Sterculia lychnophora* posses potential antibacterial activity against *E. coli* (Fig. 1) and *S. aureus* (Fig. 2) *S. typhi* and (Fig. 3), antifungal activity against *C. albicans* (Fig. 4). Whereas *S. pyogenes* and *K. pneumoniae* were found to be resistant against all extracts under study. When tested by the

Agar cup method, the cold aqueous extract showed highest antibacterial activity against *S. typhi* more than 12mm, a significant activity was recorded against *S. aureus* and *E. coli* around 8–12mm, there was the lowest activity against *C. albicans* and no activity was showed against *S. pyogenes* and *K. pneumoniae*.

Table.1 Microorganisms with strain number and suitable media at suitable incubation temperature were studied

Sr.No.	Name of the cultures	NCIM	Growth media	Incubation Temperature
1	*Escherichia coli*	ATCC10536	Nutrient agar	37°C
2	*Salmonella typhimurium*	ATCC 23564	Nutrient agar	37°C
3	*Staphylococcus aureus*	2602	Nutrient agar	37°C
4	*Streptococcus pyogenes*	2608	Nutrient agar	37°C
5	*Candida albicans*	Clinical isolates	Sabouraud agar	Room temp.
6	*Klebsiella pneumoniae*	Clinical isolates	Nutrient agar	37°C

Table.2 Antimicrobial effect of Sterculia lychnophora extracts (Zone of inhibition in mm)

Extract	*S.aureus*	*E.coli*	*S.typhi*	*S.pyogenes*	*K.pneumoniae*	*C.albicans*
Cold Water	++	++	+++	_	_	+
Hot water	_	+	_	_	_	+
Cold Methanol	++	++	_	_	_	+
Hot Methanol	++	+	+	_	_	+

Key:

- No zone of inhibition
+ Zone of inhibition upto 8mm
++ Zone of inhibition 8mm-12mm
+++ Zone of inhibition more than 12 mm

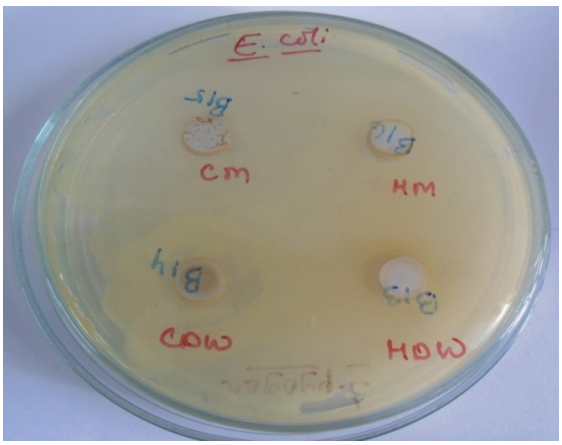

Fig.1 Zone of inhibition of bioassay of cold and hot methanol and cold and hot aqueous extract of *Sterculia lychnophora* on *E.coli*

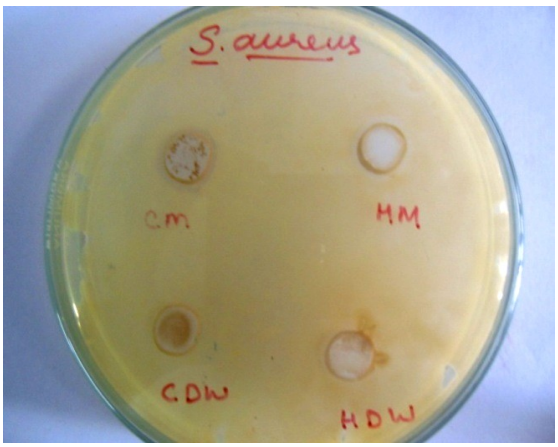

Fig.2 Zone of inhibition of bioassay of cold and hot methanol and cold and hot aqueous extract of *Sterculia lychnophora on S.aureus*

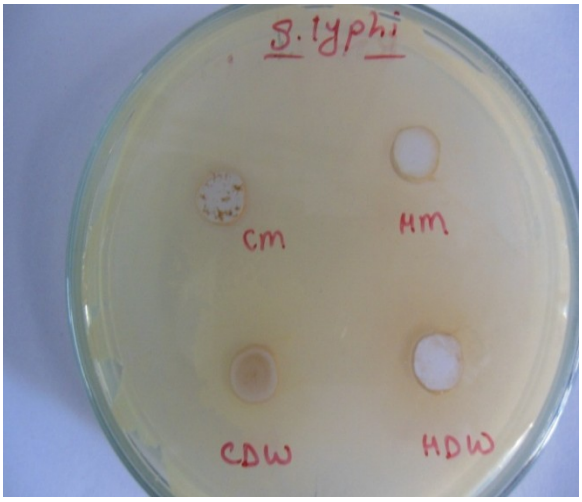

Fig.3 Zone of inhibition of bioassay of cold and hot methanol and cold and hot aqueous extract of *Sterculia lychnophora on S.typhi*

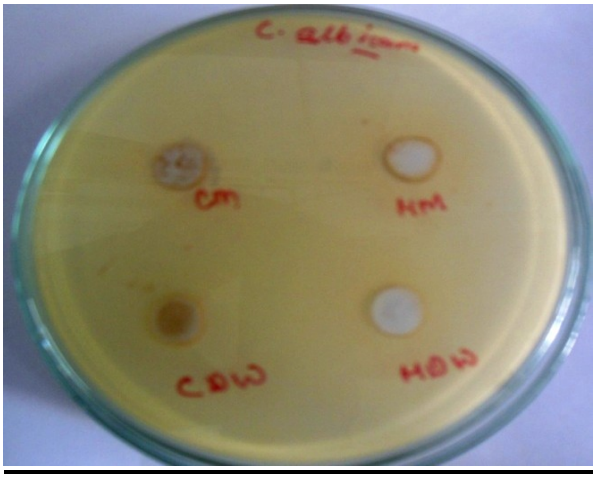

Fig.4 Zone of inhibition of bioassay of cold and hot methanol and cold and hot aqueous extract of *Sterculia lychnophora on C.albians*

Hot aqueous extract showed the activity against *E. coli* and *C. albicans* and there was no inhibition activity against *S. aureus, S. typhi, S. pyogenes* and *K. pneumoniae*. Cold methanol extract showed a significant growth against *S. aureus* and *E. coli* around 8-12mm, the lowest activity was against *C. albicans* of around 8mm and there was no growth against *S. typhi, S. pyogenes* and *K. pneumoniae*. Hot methanol extract showed a significant activity against *S. aureus* around 8-12mm, lowest activity was against *E. coli, S. typhi* and *C. albicans* around 8mm and no activity was showed against *S. pyogenes* and *K. pneumoniae*.

In conclusion, the study showed antimicrobial activity of extracts of seeds of *Sterculia lychnophora against S. aureus, E. coli* and *S. typhi* and the highest zone of inhibition was of cold water extract against *S. typhi* whereas *S. pyogenes* and *K. pneumoniae* were resistant against all extracts. Thus, the seeds of *Sterculia lychnophora* possess in vitro antibacterial and antifungal activity which can be used in treatment of infectious diseases.

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