

SCREENING OF *IN VITRO* ANTIBACTERIAL ACTIVITY OF *TECTONA GRANDIS* ON BURN PATHOGENS

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ABSTRACT

The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine is based on ethno pharmacological and taxonomic information. The frontal leaves of *Tectona grandis* (Verabaceae) are widely used in the folklore for the treatment of various kinds of wounds, especially burn wound. The present study was carried out to determine the antibacterial activity of crude chloroform extract of *Tectona grandis* leaves on pathogenic organisms isolated from infected burn patients using the disc diffusion method of sensitivity testing results obtained showed that the extract produced significant zones of lyses against all the pathogens studied. When these results were compared with those obtained using the conventional antibiotic cream namely Silver Sulphadiazine (SSD), no significant difference was observed between both zones of inhibition produced.

KEY WORDS

Antimicrobial activity, Chloroform extract, Minimum Inhibitory Concentration (MIC), *Tectona grandis*.

INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and which are used in the primary health care system (Blanks, 1998). Plants have been are as medicines for decades. India has a rich heritage of using medicinal plants such as Ayurveda, Siddha and Unani in traditional medicines besides folklore practices. The earliest mention of the medicinal uses of plants is found in the Rigveda which is one of the oldest repositories on human knowledge (Chopra, 1958).

Burns remain a huge public health issue, in terms of morbidity and long term disability throughout the world especially in developing countries (Alaghebandan, 2001). Approximately, 50-75% of hospital deaths are reported to be due to infections (Mokoddass, 1998). Burn patients are at high risk for of nosocomial infections due to multidrug- resistant

bacteria species a high proportion of which was due to gram- negative organisms (Moore, 1999). Bacterial colonization of burned and devitalized tissue is inevitable and invasive bacterial infection is still one of the major problems in the treatment of burn victims (Gnanamani, 2003). The most common pathogens causing serious infection in burn patients include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Proteus sp*, *Clostridium sp* and *Coliforms* (Lawrence, 1999).

Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plant origin have enormous therapeutic potential (Evans, 2002). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). The problem of microbial resistance is growing and the

outlook for the use of antimicrobial drug in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotics, and develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural.

Tectona grandis is commonly known as Indian Teak, and it belongs to the family Verbinaceae. The frontal leaves of the plant are widely used in folklore for the treatment of various kinds of wounds, especially burn wounds. They are also useful to treat haemostatic, depurative, anti inflammatory and vulnerary and also useful in inflammation, leprosy, skin disease, pruritus, stomatitis, indolent ulcer, haemorrhags and haemopstysis (Data base on medicinal plants used in Ayurved). The present work deals with the screening of *Tectona grandis* leaf extract for *in vitro* antibacterial activity against burn pathogens and comparing their efficacy against conventional antibiotic cream, namely Silver Sulphadiazine (SSD).

MATERIALS METHODS

Collection of plant material

Tectona grandis leaves were collected from the garden of Dr. M.G.R. University during April- May 2009, Chennai, India. The plant material was identified by Dr.K.Balakrishnan, Research Officer, central Research institute for Ayurveda and Siddha (Central Council for Ayurveda and Siddha), Arumbakkam, and Chennai. The Collected plant material was air dried under shade at room temperature, ground with hand grinder leaving a particle with size of approximately 300µm approximately.

Preparation of extract

About 500gm of powdered leaf sample were soaked in 1 liter of chloroform using soxhlet apparatus for 72 hrs. Chloroform removal carried out under pressure. A semi solid mass with a yield of 13 percent.

Preparation of test solution and disc

Test solution was prepared with known weight of crude extract, dissolved in Dimethyl Sulphoxide (DMSO). Whatman no.1 sterile filter paper disc (6 mm) were impregnated with 20 µl of this extract (corresponding to 500 and 1000 µl/ml of crude plant

extract) and allowed to dry at room temperature (Chandrasekaran , 2003).

Disc diffusion method and minimum inhibition concentration

Eight pathogenic species namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Shigella boydii*, *Salmonella typhi* and *Proteus vulgaris* were used to assess the antibacterial activity of the plant extract. These organisms were isolated from infected wound sites of patients having burn injuries. Isolation of these pathogens was carried out according to standard procedures by the microbiology division associated with Burn Ward Unit, ACS Medical College and Hospital (a unit of Dr. M.G.R. University), Chennai, India. The antibacterial effect of the chloroform extract (free from chloroform) was evaluated by disc diffusion method (Maruzzella and Henry, 1958). Silver Sulphadiazine solution at a concentration of 1mg/ml was used to compare the effect of test compounds. Solvent (DMSO) used for stabilizing these extract were kept as controls. The Minimum inhibitory concentration (MIC) of the extracts for the lyses of cells was assessed according the method of Hernandez-Perez. (1994)

RESULTS AND DISCUSSION

Disc-diffusion Method

Fig. 1 illustrate the zone of lysis by disc-diffusion method using crude chloroform extract (free from chloroform) leaves of *Tectona grandis* against eight pathogenic species isolated from burn patients on comparison with the Silver Sulphadiazine (SSD). The minimum inhibitory concentration of this extract is summarized in **Table 1**.

Sensitivity towards different burn pathogens by chloroform extract of *Tectona grandis* leaves ware in the following order viz., *Shigella boydii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhi*. The antibacterial effect of this extract was found to be comparable to the antibacterial activity exhibited by conventional antibacterial cream, Silver Sulphadiazine (SSD). On considering antibacterial activity of *Tectona grandis* leaf extract exhibit a better antibacterial

activity against pathogens from burn injuries. Regarding *Tectona grandis*, though it showed inhibition irrespective of the species studied, the efficiency is found to be very much less in *Salmonella typhi* MIC studies. It reveals that about 0.125 ± 0.02 mg/ml of *Tectona grandis* leaf extract was required for inhibition of growth for *Proteus vulgaris* to other species (0.030- 0.07 mg/ml). The requisite of this higher concentration is the reason for the three fold decrease in the zone of lyses activity where the applied concentration may be able to produce increased zone of lyses as given in **Fig.1**. Shah *et al* (1995) reported the *Tectona grandis* containing tannin, which are used as anti inflammatory agents and also used topically for the treatment of burns. Lipid Peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the variability collagen fibrils, increasing the strength of collagen fibrils by an increase in the circulation, thereby, preventing the cell damage and promoting the DNA synthesis. Antioxidants such as metronidazole, vitamin C, vitamin E have been shown to promote wound contraction

and epithelization (Rao & Ghosh, 1997). The antioxidant property of the *Tectona grandis* leaves, conferred upon by the presence of high amounts of tannin may also be responsible for pro-healing action of the extract (Mrityunjoy Majumdar, 2007).

The result obtained from the present study suggested that these plant extract possess significant antibacterial property. *Tectona grandis* leaves active compounds can well be exploited for burn wound management. As infections being a major cause of morbidity and mortality in burn patients, this herbal extracts may prevent infection that leads to high risk of sepsis, and thereby prevents the prolongation of inflammatory phase.

Further study on the fractionation of active components and the mutual effect this plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing. Since *Tectona grandis* appears to be most promising, bio assay guided fractionation, is currently underway with a goal of elucidating their active antimicrobial compounds.

Fig.1. Zone of lysis measured in mm by disc-diffusion assay for chloroform extract of *Tectona grandis* on eight burn pathogens on comparison with Silver Sulphadiazine (SSD).

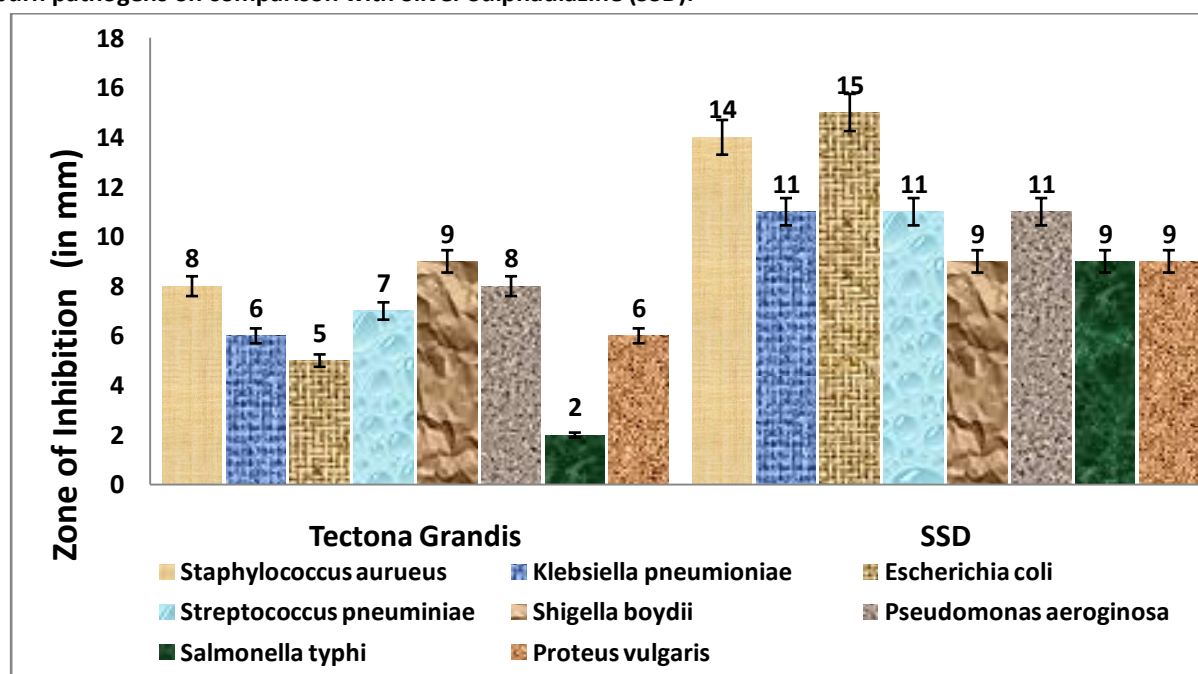


Table 1: Minimum inhibitory concentration (MIC) exhibited by crude chloroform extracts of *Tectona grandis* (Concentration: mg/ml).

S.no	organisms isolated from infected burn patients	Crude chloroform extract of <i>Tectona grandis</i>
1.	Staphylococcus sp.	0.06 ± 0.01
2.	Klebsiella sp.	0.07 ± 0.02
3.	E. coli	0.065 ± 0.01
4.	Streptococcus sp.	0.075 ± 0.02
5.	Shigella sp.	0.030 ± 0.01
6.	Pseudomonas sp.	0.065 ± 0.02
7.	Salmonella sp.	0.125 ± 0.02
8.	Vibrio sp.	0.07 ± 0.03

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