www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 519-523, Jan-Mar 2010

SYNERGISTIC *IN VITRO* ANTIBACTERIAL ACTIVITY OF *TECTONA GRANDIS* LEAVES WITH TETRACYCLINE

K G PURUSHOTHAM*[1], P ARUN[1], J JOHNSY JAYARANI[1], R VASNTHAKUMARI[1], L SANKAR[2], BIJJAM RAVIPRAKASH REDDY[1]

[1]Department of Industrial, Biotechnology,

[2]Department of Chemistry,

Dr.M.G.R.Educational and Research Institute, Dr.M.G.R University, Maduravoyal.

Chennai-600 095, Tamil Nadu, India.

Corres.author:purushoth_13@rediffmail.com
Ph.No.0988428831

ABSTRACT: The frontal leaves of *Tectona grandis* (Verabinaceae) are widely used in the folklore to treat various kinds of infections. The aim of this study was, to formulate new cost effective antimicrobial agent for multi drug resistant organisms, based on the synergistic activity of Tetracycline with methanolic extract of *Tectona grandis*. The Minimum Inhibition Concentration (MIC) of methanolic extract in combination with Tetracycline using 9 different Gram-positive and Gram-negative bacteria was found to be around 62.5 µg/ml -2000 µg/ml. The synergistic activity was varied using Kirby-Bauer method. It shows maximum synergistic activity against different bacteria both Gram-positive and Gram-negative species. The higher synergistic rate was attained against *Salmonella typhimurium*(MTCC 98) , *Klebsiella pneumonia* (MTCC 432), and lowest synergistic shows against *Pichia pastoris* (MTCC 34), *Escherichia coli,* (MTCC 729). No synergistic activity was observed in *Citrobacter freondii* (MTCC 1658)

KEY WORDS: Antibacterial activity, Minimum Inhibition Concentration (MIC), *Tectona grandis,* Tetracycline, Synergism.

INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and are still use in the primary health care system[4]. Plants based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occurs. Antimicrobials of plant origin have enormous therapeutic potential[17]. Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents[18]. Different extracts from traditional medicinal plants have been tested. Many reports have showd the effectiveness of traditional herbs against microorganisms; as a result, plants are one of the bedrocks for modern medicine to attain new principles[6]. Since the majority of bacteria are resists to many antibiotics, only ampicillin and/or chloramphenicol and /or oxytetracycline are used in the synergism assay. This was because the resistance to at least one of these drugs was common in all the bacteria tested[15]. The synergistic effect from the association of antibiotics with plant extracts against resistant bacteria leads to new choice for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment. There fore, the present study was undertaken for the first time to investigate synergistic activity of methanol extract of *Tectona grandis* with Tetracycline.

Tectona grandis is a large deciduous tree 10-12 meter tall; branch lets 4-angled, density clothed with yellowish grey tomentum. Leaves opposite, elliptic or obovate, 30-50 x 15-20 cm, cuneate at base, entire or crenulate, acute or acuminate, rough and glabrous above, stellate, grey to tawny tomentose beneath[5]. It commonly known as Indian teak and it belongs to family verabinaceae. Lapachal, a napthoquinone

isolated from *Tectona grandis* is reported to have anti ulcer[8] and nitric oxide scavenging activity[11].

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 Ayurvda and Siddha (Central Council for Ayurveda and Siddha), Arumbakkam, and Chennai. Collected plant material was air dried under shade at room temperature, ground with hand grinder having particle size 300µm approximately

Preliminary phytochemical screening

The preliminary phytochemical screening of *Tectona grandis* was carried out from the various phytoconstituents using standard procedures [7]. The following solvents were used for the study, petroleum ether, ethyl acetate, chloroform, ethanol & methanol. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract revels the presence of alkaloids, flavonoids, tannins.

Preparation of crude extract

Weighed quantities of coarsely powdered leaves of *Tectona grandis* were placed in maceration flask and added with sufficient quantity of methanol. Complete maceration takes place for about 72 hours, with occasional shaking during first 6 hours[12]. After 72 hours, the men strum was collected and evaporated to obtain the dried extract.

Bacterial strains

The different bacterial strains used for study were *Klebsiella pneumonia* (MTCC 432), *Psedomonas aeruginosa* (MTCC 1688), *Proteus mirabilis* (MTCC 425), *Escherichia coli,* (MTCC 729), *Salmonella typhimurium*(MTCC 98), *Citrobacter freondii* (MTCC 1658), *Serratia marcescens* (MTCC 97), *Pichia pastoris* (MTCC 34),and *Streptococcus species* (MTCC 389).**(Invoice no MTCC/07/8/4836)**

Minimum Inhibitory Concentration (MIC)

A series of culture tubes[8] were prepared all containing the same volume of the medium inoculated with test microorganisms. The lowest concentration of sample at which the subculture from test dilution yielded no viable organisms was recorded as minimum bactericidal concentration[16]. Decreasing concentration of drug was added to the tubes usually a step wise dilution (2-fold serial dilutions) was used starting from highest to lowest concentrations. One tube was lift without drug to serve as positive control and other without drug and inoculums to serve as negative control. The cultures were incubated at a temperature optimal for growth of the test organisms and a period of time sufficient for growth for at least 10-15 generators (usually 24 hours for bacteria at 37°c). The tubes were inspected visually to determine the growth of organisms by the presence of turbidity and the tubes in which antibiotic is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as Minimum Inhibitory Concentration(MIC) of the extract. In experimental terms Minimum Inhibitory Concentration (MIC) is the concentration of the drug present in the last clear tube that is the tube having the lowest antibiotic concentration in which growth is not observed.

Synergistic activity

The synergistic activity study was calculated by combining with the standard antibiotics Tetracycline by means of Cup plate method (Kirby & Bauer technique) using two wells in a plate methanolic plant extract of *Tectona grandis* 125 µg/ml was used in combination with Tetracycline 62.5 µg/ml. The distance between the two wells was maintained as standard of about 0.8 cm then incubated at the standard conditions for 24 hours at 37°c and the zone diameters was measured in the second day[2].

RESULTS

The preliminary phytochemical screening reveals the presence of flavonoids, alkaloids, tannins, anthraquinones and napthaquinones. The MIC was carried out for Tetracyclin alone and then for the methanolic extract of *Tectona grandis* and finally combination of Tetracycline and methanolic extract of *Tectona grandis* (1:1). The results were presented in Table I. The MIC values were found to be less with Tetracycline alone and it was found to be still lesser with the methanolic extract of *Tectona grandis*. However, the MIC was found to be the least with combination of Tetracycline and methonolic extract of *Tectona grandis*. Moreover, the therapeutic efficacy was found to be higher even in low concentration. This clearly exhibits the advantages of administering the combinations of Tetracycline and methanolic extract of *Tectona grandis* over the other two individual forms coupled with enhanced synergistic activity.

The antimicrobial activities of methanolic extract of *Tectona grandis* on various strains were confirmed and synergism was possible with the antimicrobial drug tested. Tetracycline presented good synergism with methonolic extract of *Tectona grandis*. In these findings, *Salmonella typhimurium*(MTCC 98) and *Klebsiella pneumonia* (MTCC 432), shows higher synergism, indicates higher zone diameter (36), lowest synergism was observed in *Pichia pastoris* (MTCC 34) and *Escherichia coli,* (MTCC 729),. No synergistic activity was observed in the *Citrobacter freondii* (MTCC 1658), Out of 9 different Gram-negative and Gram-positive tested, maximum shows synergistic activity against these microorganisms (Table II).

Table 1: Minimum inhibitory concentration (MIC) of Methanolic extract of *Tectona grandis* leaves

Microorganisms	MIC of TC (µg/ml)	MIC of TG (µg/ml)	MIC of TG+TC (1.1) (µg/ml)
Psedomonas aeruginosa (MTCC 1688)	≥500	≥1000	62.5
Klebsiella pneumonia (MTCC 432),	≥1000	≥1000	250
Proteus mirabilis (MTCC 425	≥500	≥1000	125
Salmonella typhimurium(MTCC 98),	≥500	≥500	250
Escherichia coli, (MTCC 729	≥500	≥1000	125
Serratia marcescens (MTCC 97	≥500	≥1000	62.5
Citrobacter freondii (MTCC 1658),	≥2000	≥2000	1500
Pichia pastoris (MTCC 34),	≥500	≥1000	125
Streptococcus species (MTCC 389)	≥250	≥500	250

TC=Tetracycline, TG=Methanolic extract of *Tectona grandis*, TG+ TC=Methanolic extract of *Tectona grandis* + Tetracycline

Table 2: Synergistic activity of Methanolic extract of *Tectona grndis* leaves

Microorganism	Zone of inhibition (mm)		
	TC	TG	TG+TC
Psedomonas aeruginosa (MTCC 1688)	20	18	22
Klebsiella pneumonia (MTCC 432),	21	15	36
Proteus mirabilis (MTCC 425	23	22	28
Salmonella typhimurium(MTCC 98),	29	26	36
Escherichia coli, (MTCC 729	17	14	22
Serratia marcescens (MTCC 97	20	25	27
Citrobacter freondii (MTCC 1658),	09	0	0
Pichia pastoris (MTCC 34),	20	18	22
Streptococcus species (MTCC 389)	24	24	30

TC=Tetracycline, TG=methanolic extract of *Tectona grandis*, TG+TC=methanolic extract of *Tectona grandis*+ Tetracycline

DISCUSSION

The objective of antimicrobial activity was to analyze past, present and future of medicinal plants to suggest as fundamental the research on plant extract mechanism of action, interactions with antibiotics or with other medicinal plants. Research on synergism is very limited and few studies have been reported using Kirby and Bauer method[2] and moreover falvonoids exhibit a broad spectrum of biological activity including antiviral activity[13].

The results of the synergism study depicted that the protein synthesis inhibitors were those that presented stronger synergistic effect together with folic acid and bacterial cell wall synthesis inhibitors whereas inhibitors of the nucleic acid synthesis showed weak synergism with plant extracts. Further studies on the chemical characteristics of extracts and active components should be carried out since only crude extracts and their dry weight have been used in MIC determination expressed in mg/ml and synergism assays. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism

profile between plant extracts and inhibitors of protein synthesis; however the understanding of synergism mechanism is fundamental to development of pharmacological agents to treat disease caused by different microbes using medicinal plants[1].

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumonia* is the most important member of the *Klebsiella* genus of enterobacteiaceae and its emerging as an important cause of neonatal nosocomial infection[10]. Escherichia coli causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients[2]. Infection caused by salmonella typhi is a serious public health problem in developing countries and represents a constant concern for the food industry[14]. The demonstration of activity against both Gram-negative and Gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be broad spectrum of activity.

Thus, the researchers to investigate the synergistic capacity of plants or other natural products, independent of the antimicrobial activity they have. There fore the results of the present study seems to be promising and may enhance the natural products uses, showing the potentiality of *Tectona grandis* in the treatment of various infectious diseases caused by bacteria. Further studies on the chemical characteristics of extract and active components should be carried out for the plant and its antimicrobial property. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and the inhibitions of protein synthesis, however, the understanding of synergism mechanism is fundamental to development of pharmacological agents to treat disease by various bacteria using medicinal plants[2].

ACKNOWLEDGEMENT

The author is thankful to the staff members from ACS Medical College & Hospital, Chennai, India, and sincerely acknowledges to Dr. K.Balakrishnan Research Officer, central Research institute for Ayurvda and Siddha (Central Council for Ayurveda and Siddha), Arumbakkam, and Chennai. For identification of plant material

REFERENCES

1. Arthanari Saravana Kumar, Venkateswaran K, Vanitha J, Saravana V S, Ganesh M, Vasudevan M, Sivakumar T "Synergistic activity of methanolic extract of *Thespeia populnea* flower with oxytetracycline" Bangladesh J Pharmacol.,2009;4:13-16.
2. Betony J E C, Mantouani R P, Barbosa L N, Sasti L C D, Fernender A "Synergism between plants extract and antimicrobial drugs used on *Stphylococcus aureus* diseases" Mem Inst Oswaldo Cruz,Rio De Janerio.,2006;101:387-390.
3. Black J G, Microbiology "Princeples and application." New York, Prentice Hall, 1996; p260.
4. Blanks T S, Brown B, Cosgrave J, Woody V, Bentley, Sullivan N. "The Body Shop Book of wellbeing Mind, Body and Soul", Ebury Press London,1988; pp173-192.
5. Data base on medicinal plants used in aurveda Vol: 5 pp: 295-303.
6. Evans C E, Banso A, Samuel O A"Efficacy of some nupe medicinal plants against *Salmonella typhi*: an *in vitro* study" J.Ethanopharmocol., 2002;80:21-24.
7. Evans W C, "Trees and Evans pharmacognosy" London, Balliere, tindall., 1996;p388.
8. Ferreira M J U, Ascenso J R, Valderia L, Duarte A, Frade J P, Freitas G "Evaluation of antiviral and antimicrobial activities of triterpenes isolated from *Euphorbia segetalis*" Nat Prod Res.,2003; 17:375-380.
9. Goel R K, Pathak N K, Biswas M, Pandey V B, Sanyal A K "Effect of lapachol, a naphthaquinone isolated from *Tectona gransis*, on experimental peptic ulcer and gastric sectetion" J. Pharm. Pharmacol.,1987;39(2):138-140.
10. Gupta P, Murali P, Murali M V, faridi M M A, Kaul P B, Ramachandran V C, Talwar V "*Klebsiella* septicaemia in neonates" Ind J Paediatr.,1993;60:565-572.
11. Jagetia G C,Baliga M S "The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: a preliminary study" J. Med. Food.,2004;7(3):343-348.
12. Kumar A S, Mazumdar, Saravanan V S A "Antihyperlipidemic activity of *Camellia senensis* leaves in Triton-WR 1339 induced Albino rats" Phcog Mag., 2008;4:60-64.
13. Li S, Lo C Y, Ho C T "Hydroxylated polymethoxy flaones and methylated falvonoids in sweet orange peel" J Agric Food Chem.,2006;54:4176-4185.
14. Mastroeni P "Immunity to systemic Salmonella infections" Curr Mol Med., 2002; 2:393-406.

15. Nascimento G G F, Locatelli J, Freitas P C, Silva G L. "antibacterial activity of plant extracts and phytochemical on antibiotic-resistant bacteria" Braz J Microbiol., 2008; 31:247-256.
16. Nazaruk J, Jakoniuk P "Flavonoid composition and antimicrobial activity of *Crisium rivulare* flowers" J.Ethanopharmocol., 2005;102:208-212.
17. Salau A O, Odeleye O M "Antimicrobial activity of *Mucuna pruriens* on selected bacteria" African J. Biotechnol., 2007; 6(18):2091-2092.
18. Werner F, Okemo P, Ansorg R "Antibactrial activity of East African Medicinal plants" J.Ethanopharmocol., 1999;60:79-84.

*****