SYNERGISM BETWEEN METHANOLIC EXTRACT OF *SESBANIA GRANDIFLORA* (FABACEAE) FLOWERS AND OXYTETRACYCLINE.

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Summary

The object of this study was to formulate new, cost effective anti-microbial combination for multidrug resistant diseases based on the synergistic activity of oxytetracycline with methanolic extract of *Sesbania grandiflora* (Fabaceae) a medicinal plant common in South India. The MIC of methanolic extracts in combination with oxytetracycline using 12 different both gram positive and gram negative bacteria's was found to be around (62.5 µg/ml to 1000 µg/ml). The synergistic activity was verified for the methanolic extract of *Sesbania grandiflora* using Kirby and Bauer techniques. The different microorganisms used for our study were *Shigella sonei* (ATCC 29930), *Escherichiae coli* (ATCC11229), *Shigella boydii* (ATCC8700), *Rhodococcus terrae* (NCIM 5126), *Micrococcus flavum* (NCIM 2984), *Flavobacterium devorans* (NCIM 2581), *Brevibacterium leuteum* (ATCC 15830), *Bacillus lichenformis* (NCIM 2468), *Salmonell typhii* (ATCC 13313), *Klebsiella pneumonia* (ATCC 11229), *Micrococcus leuteus* (ATCC 9341) and *Shigella flexneri* (NCIM 4924) the inhibitory zones were measured in millimetres. 83.3% shows synergistic activity against all 12 different bacterias both gram positive and gram negative species. The highest synergism rate was attained against *Shigella boydii* (ATCC8700).

Key words: *Sesbania grandiflora*, Methanolic extract, Synergistic activity, Inhibitory zones

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Introduction

Sesbania grandiflora L. (Fabaceae) is popularly known as "Basna" is an ornamental plant and is found in plains of western Himalayas to Sri Lanka (1). *Sesbania grandiflora* is a folk remedy for bruises, catarrh, dysentry, eyes, fevers, headaches, small pox, sores, sore throat and stomatitis (2). It is a small erect quick growing short-lived, soft-wooded tree to 10m tall, sparsely branched. Bole straight and cylindrical, the wood white and soft. Bark light grey, corky, deeply furrowed. Leaves pinnate, 15-30cm long, with 16-30 pairs of linear oblong leaflets. Racemes 2.5cm long. Flowers 2-4, white to pink, pendulous the corolla 7-9cm long, pods 50-60cm long. Bark, leaves, gums and flowers are considered medicinal. The astringent bark was used in treating small pox and other eruptive fevers. The juice from the flowers is used to treat headache, head congestion of stuffy nose. Rheumatic swellings are poulticed or rubbed with aqueous decoctions of the powdered roots of the red flowered variant. Ayurvedics, believing the fruits to be alexeteric, laxative and intellectually stimulating, prescribe them for anaemia, bronchitis, fever, pain, thirst, ozoena and Quartan fever. Yuani consider the tonic levels useful in biliousness, fever and nyctalopia. Indians apply the roots in rheumatism, the juice of the leaves and flowers for headache and nasal catarrh (3). In Amboina, flower juice is squeezed in to the eye to correct dim vision. The bark is used in infusions for small pox. Cambodians consider the flowers emollient and laxative, the bark for diarrhea, dysentry and paludism.

Malayans apply crushed leaves to sprains and confusions. They gargle with the leaf juice to cleanse the mouth and throat. In small doses, the bark is used for dysentry and sprue, in large doses, laxative, in still larger doses, emetic. Pounded bark is applied to scabies. Philippines use the pounded bark for hemottysis. The powdered bark is also recommended for ulcers of the mouth and alimentary canal. In java, the bark is used for thrush and infantile disorders of the stomach. Leaves are chewed to disinfect the mouth and throat (4).

Materials and Methods

Plant material

The plant materials were collected during April-May 2007 from tropical areas of Western Ghats regions of Erode and Nagercoil and then shade dried at room temperature. The Plant material were identified by G.S.R.Murthy, Joint Director at Botanical survey of India (BSI) Coimbatore, India and a voucher specimen (SC 5/23) was deposited in Herbarium of Laboratory of Botany, Coimbatore, Tamilnadu, India.

Chemicals

All chemicals and solvents used in this experiment were of analytical grade obtained from BDH, Poole, UK.

Preliminary phytochemical screening

The preliminary phytochemical screening of *Sesbania grandiflora* (SG) was carried out for the decoction of various phytoconstituents using standard procedures (5).The following solvents were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins, triterpenes, gums and mucilage's.

Preparation of crude extract

Weighed quantities of coarsely powdered flowers of SG were placed in maceration flask and added with sufficient quantity of methanol. Complete maceration takes place for about 24 hrs, with occasional shaking during first 6 hours (6). After 24 hours, the menstrum was collected and evaporated to obtain the dried extract (68%).

Bacterial strains
The different bacterial strains used for our study were *Shigella sonei (ATCC* 29930*), Escherichia coli* (ATCC11229), *Shigella boydii* (ATCC8700)*, Rhodococcus terrae* (NCIM 5126), *Micrococcus flavum* (NCIM 2984*), Flavobacterium devorans* (NCIM 2581), *Brevibacterium leuteum* (ATCC 15830), *Bacillus lichenformis* (NCIM 2468*), Salmonell typhii* (ATCC 13313*), Klebsiella pneumoniae* (ATCC 11229), *Micrococcus leuteus* (ATCC 9341*)* and *Shigella flexneri* (NCIM 4924).

Minimum inhibitory concentration
A series of culture tubes (micro dilution assays) (7) were prepared all containing the same volume of 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 (8). Decreasing concentration of drug was added to the tubes usually a step wise dilution (two fold serial dilutions) was used starting from highest to lowest concentrations. One tube was left without drug to serve as positive control and other without drug and inoculum to serve as negative control. The cultures were incubated at a temperature optimal for growth of the test organism and a period of time sufficient for growth for atleast 10-15 generators (usually 24hrs 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 MIC of the extract. In experimental terms 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 oxytrtracycline by means of cup plate method (Kirby and Bauer technique) using two wells in a plate methanolic plant extract of SG 125µg/ml was used in combination with oxytetracycline 62.5µg/ml. The distance between the two wells was maintained as standard of about 0.8 cm then incubated at the standard conditions for 24 hrs at 37°C and the zone diameters was measured in the second day (9).

Results

Preliminary phytochemical screening
The preliminary phytochemical screening reveals the presence of flavonoids alkaloids, tannins and anthraquinone glycosides.

Minimum inhibitory concentration
The minimum inhibitory concentration was carried out for oxytetracycline alone and then for the extract of *Sesbania grandiflora* and finally combination of oxytetracycline and methanolic extract of *Sesbania grandiflora* (1:1). (Table 1). The MIC was found to be less with methanolic extract of *Sesbania grandiflora* alone and it was found to be still lesser with the oxytetracycline. However, the MIC was found to be the least with combination of oxytetracycline and methanolic extract of *Sesbania grandiflora*. Moreover, the therapeutic efficacy was found to be higher even in low concentration. This clearly exhibits the advantage of administering the combination of oxytetracycline and methanolic extract of *Sesbania grandiflora* over the other two individual forms coupled with enhanced synergistic activity.

Table 1: Minimum Inhibitory Concentration (MIC) of methanolic extract of *Sesbania grandiflora* flowers.

Microorganisms	MIC of O (µg/ml)	MIC of E (µg/ml)	MIC of EO (1:1) (µg/ml)
Rhodococcus terrae (NCIM 5126)	≥500	≥1000	62.5
Shigella sonei (ATCC 29930)	≥1000	≥1000	125
Salmonella typhi (NCIM 2479)	≥500	≥1000	125
Flavobacterium devorans (NCIM 2581)	≥250	≥500	250
Micrococcus flavus (NCIM 2376)	≥1000	≥1000	250
Brevibacterium leuteus (NCIM 2923)	≥500	≥1000	125
Shigella flexneri (NCIM 4924)	≥500	≥1000	250
*Shigella boydii (*ATCC 8700)	≥1000	≥1000	500
Escherichia coli (ATCC 11775)	≥500	≥1000	62.5
Bacillus licheniformis (NCIM 2468)	≥1500	≥1500	1000
Klebsiella pneumonia (ATCC 13883)	≥500	≥1000	125
Micrococcus leuteus (ATCC 2984)	≥2000	≥2000	1500

MIC- Minimum Inhibitory Concentration. O= Oxytetracycline, E = Methanolic extract of ***Sesbania grandiflora***, EO= Methanolic extract of ***Sesbania grandiflora*** + Oxytetracycline.

Synergistic activity

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 .Where as 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 diseases caused by different microbes using medicinal plants. Among the twelve different bacteria's used *Micrococcus leuteus* and *Brevibacterium leuteum* shows negative result in synergistic study the remaining bacteria's shows positive response of synergism. Out of 12 different bacterias both gram positive and gram negative tested only 83.3% shows synergistic activity against these microorganisms (Table 2).

Table 2: Synergistic activity of methanolic extract of *Sesbania grandiflora* flowers

Microorganisms	Zone of Inhibition (mm)		
	O	**E**	**EO**
Rhodococcus terrae (NCIM 5126)	20	18	20
Shigella sonei (ATCC 29930)	21	15	34
Salmonella typhi (NCIM 2479)	23	26	32
Flavobacterium devorans (NCIM 2581)	24	24	32
Micrococcus flavus (NCIM 2376)	23	22	28
Brevibacterium leuteus (NCIM 2923)	12	0	0
Shigella flexneri (NCIM 4924)	28	30	34
*Shigella boydii(*ATCC 8700)	29	26	37
Escherichia coli (ATCC 11775)	22	18	26
Bacillus licheniformis (NCIM 2468)	25	20	27
Klebsiella pneumonia (ATCC 13883)	17	14	20
Micrococcus leuteus (ATCC 2984)	09	0	0

O=Oxytetracycline, E = Methanolic extract of *Sesbania grandiflora*, EO= Methanolic extract of *Sesbania grandiflora* + Oxytetracycline.

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 and moreover flavonoids exhibit a broad spectrum of biological activity including antiviral activity (10). The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *klebsiella pneumoniae* is the most important member of the klesiella genus of enterobacteriaceae and its emerging as an important cause of neonatal nosocomial infection (11). *Escherichia coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients (12). Infection caused by *salmonella typhi* is a serious public health problem in developing countries and represents a constant concern for the food industry (13). 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. Therefore the results of the present study seem to be promising and may enhance the natural products uses, showing the potentiality of *Sesbania grandiflora* 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.

In the present study, the antimicrobial activities of methanolic extract of *Sesbania grandiflora* on various strains were confirmed and synergism was possible with the antimicrobial drug tested. Oxytetracycline presented good synergism with methanolic extract of *thespesia populnea*. In these findings, *Shigella boydii* shows higher synergism, indicates higher zone diameter (37mm), lowest synergism was observed in *Rhodococcus terrae* and *Klebsiella pneumonia* (20mm). No synergistic activity was observed in *Brevibacterium leuteum* and *Micrococcus luteus*.

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 diseases by various bacteria using medicinal plants (9).

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