BJP

Bangladesh Journal of Pharmacology

Research Article

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A journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2009; 4: 13-16
ISSN: 1991-0088

Synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline

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Article Info

Received: 8 July 2008
Accepted: 4 August 2008
Available Online: 9 August 2008

DOI: 10.3329/bjp.v4i1.958

Cite this article:
Kumar AS, Venkateshwaran K, Vanitha J, Saravanan VS, Ganesh M, Vasudevan M, Sivakumar T. Synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline. Bangladesh J Pharmacol. 2009; 4: 13-16.

Abstract

The object of this study was to formulate new, cost effective antimicrobial combination for multidrug resistant diseases based on the synergistic activity of oxytetracycline with methanolic extract of *Thespesia populnea* (Malvaceae) a medicinal plant common in South India. The minimum inhibitory concentration (MIC) of methanolic extracts in combination with oxytetracycline using 12 different gram positive and gram negative bacteria was found to be around (62.5 µg/mL to 1000 µg/mL). The synergistic activity was verified using Kirby and Bauer techniques. 83.3% shows synergistic activity against all 12 different bacteria both gram positive and gram negative species. The highest synergism rate was attained against *Shigella boydii* (ATCC8700).

Introduction

Thespesia populnea (Malvaceae) is a large tree found in tropical regions and coastal forests of India. The bark, leaves, and flowers are useful in cutaneous infections such as scabies, psoriasis, eczema, ringworm, guinea worm and the leaves of this plant used as anti-inflammatory for poultice as a folk medicine (Chang et al., 2002). Various parts of *T. populnea* are found to possess useful medicinal properties, such as antifertility, antimicrobial, anti-inflammatory, anti-oxidant, purgative and hepatoprotective activity (Shirwaikarkumar et al., 1995). Since the majority of bacteria were resistant to many antibiotics, only ampicillin and/or chloramphenicol and/or oxytetracycline were used in the synergism assays. This was because the resistance to at least one of these drugs was common in all the bacteria tested (Nascimento et al., 2000). The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices 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. Therefore, the present study was undertaken for the first time to investigate synergistic activity of methanol extract of *T. populnea* with oxytetracycline.

Materials and Methods

Plant material

The plant materials were collected during April-May 2006 from tropical areas of Western Ghats regions of Erode and Nagercoil and then shade dried at room temperature. The plant materials 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.

Preliminary phytochemical screening

The preliminary phytochemical screening of *T. populnea*

was carried out for the decoction of various phytoconstituents using standard procedures (Evans, 1996). 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 mucilages.

Preparation of crude extract

Weighed quantities of coarsely powdered flowers of *T. populnea* were placed in maceration flask and added with sufficient quantity of methanol. Complete maceration takes place for about 24 hours, with occasional shaking during first 6 hours (Kumar et al., 2008). 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* (ATCC 11229), *Shigella boydii* (ATCC8700), *Rhodococcus terrae* (NCIM 5126), *Micrococcus flavum* (NCIM 2984*), Flavobacterium devorans* (NCIM 2581), *Brevibacterium leuteum* (ATCC 15830), *Bacillus licheniformis* (NCIM 2468*), Salmonella typhi* (ATCC 13313*), Klebsiella pneumoniae* (ATCC 11229), *Micrococcus leuteus* (ATCC 9341*)* and *Shigella flexneri* (NCIM 4924).

Minimum inhibitory concentration (MIC)

A series of culture tubes (microdilution assays) (Ferreira et al., 2003) 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 (Nazaruk and Jakoniuk, 2005). 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 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 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 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 oxytetracycline by means of cup plate method (Kirby and Bauer technique) using two wells in a plate methanolic plant extract of *T. populnea* 125 µg/mL was used in combination with oxytetracycline 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 (Betoni et al., 2006).

Results

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

The MIC was carried out for oxytetracycline alone and then for the extract of *T. populnea* and finally combination of oxytetracycline and methanolic extract

Table I

Minimum inhibitory concentration (MIC) of methanolic extract of *T. populnea* 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

O = Oxytetracycline, E = Methanolic extract of *T. populnea*, EO = Methanolic extract of *T. populnea* + oxytetracycline

of *T. populnea* (1:1). The results were presented in Table I. The MIC values were found to be less with oxytetracycline alone and it was found to be still lesser with the methanolic extract of *T. populnea*. However, the MIC was found to be the least with combination of oxytetracycline and methanolic extract of *T. populnea*. 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 *T. populnea* over the other two individual forms coupled with enhanced synergistic activity.

The antimicrobial activities of methanolic extract of *T. populnea* on various strains were confirmed and synergism was possible with the antimicrobial drug tested. Oxytetracycline presented good synergism with methanolic extract of *T. populnea*. In these findings, *Shigella boydii* shows higher synergism, indicates higher zone diameter (36 mm), lowest synergism was observed in *Rhodococcus terrae* and *Klebsiella pneumonia* (22 mm). No synergistic activity were observed in *Bacillus licheniformis* and *Micrococcus luteus*. Out of 12 different bacterias both gram positive and gram negative tested only 83.3% shows synergistic activity against these microorganisms (Table II).

Table II

Synergistic activity of methanolic extract of *T. populnea* flowers

Microorganisms	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
Microorganisms	O	E	EO
Rhodococcus terrae (NCIM 5126)	20	18	22
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)	25	20	27
Shigella flexneri (NCIM 4924)	28	30	34
Shigella boydii (ATCC 8700)	29	26	36
Escherichia coli (ATCC 11775)	22	18	26
Bacillus licheniformis (NCIM 2468)	12	0	0
Klebsiella pneumonia (ATCC 13883)	17	14	22
Micrococcus leuteus (ATCC 2984)	09	0	0

O = Oxytetracycline, E = Methanolic extract of *T. populnea*, EO = Methanolic extract of *T. populnea* + oxytetracycline

Discussion

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 diseases caused by different microbes using medicinal plants.

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 (Betoni et al., 2006) and moreover flavonoids exhibit a broad spectrum of biological activity including antiviral activity (Li et al., 2006).

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 (Gupta et al., 1993). *Escherichia coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients (Black, 1996). Infection caused by *Salmonella typhi* is a serious public health problem in developing countries and represents a constant concern for the food industry (Mastroeni, 2002). 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 seems to be promising and may enhance the natural products uses, showing

the potentiality of *T. populnea* 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 diseases by various bacteria using medicinal plants (Betoni et al., 2006).

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