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ENHANCEMENT OF THE ANTIBIOTIC ACTIVITY AGAINST DRUG RESISTANT STRAINS OF *ESCHERICHIA COLI* BY SIDDHA DRUG MATHAN THAILAM

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ABSTRACT

This is the first report testing the antibiotic resistance-modifying activity of a Siddha formulation. In this study Mathan thailam (a traditional Siddha formulation used for skin diseases), Ethanolic extract of Datura metel and chlorpromazine were tested for their antimicrobial activity alone and in combination with conventional antibiotics against strains of *Escherichia coli.* The growth of two *Escherichia coli* strains tested was not inhibited by the Mathan thailam and ethanolic extract Datura. The minimum inhibitory concentration and minimal bactericidal concentration values were >1mg/ml for both strains of *Escherichia coli* tested. A potentiating effect of this extract on gentamicin was demonstrated. Similarly, there was a potentiating effect of chlorpromazine on kanamycin, amikacin and tobramycin, indicating the involvement of an efflux system in the resistance to these aminoglycosides. Results indicate that Mathan thailam could be used as resistance-modifying agent, such as in the case of gentamicin, making it a new weapon against bacterial resistance to antibiotics, as with chlorpromazine.

Keywords: Siddha drug, Mathan thailam, *Datura metel*, Antimicrobial activity, Enhancement of antibiotic activity, Drug resistant *Escherichia coli*.

INTRODUCTION

Since time immemorial, Herbal medicines are contributing significantly towards the healthcare of human society. Herbal medicines resulted out of therapeutic experiences of generations after generations through practices of physicians of indigenous systems of medicine over hundreds of years. In recent years, among the world population, there is an increasing trend towards the usage of herbal medicines especially Siddha medicines (Indian System of Medicine) which may be probably due to the side effects and enormous cost involved in modern medicines. And with an increased incidence of resistance to antibiotics, herbal medicines and natural products from plants could be interesting alternatives.[1,2] So it is necessary to scientifically evaluate these medicines and their phytoconstituents.

Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate this efficacy.[3-5] Many plants have been evaluated not only for direct antimicrobial activity, but also as a resistance-modifying agent.[6,7] Several chemical compounds, synthetic or from natural sources, such as the phenothiazines, and natural products, have direct activity against many species of bacteria, enhancing the activity of a specific antibiotic,

reversing the natural resistance of specific bacteria to given antibiotics, promoting the elimination of plasmids from bacteria such as *Escherichia coli,* and inhibiting transport functions of the plasma membrane in regard to given antibiotics. The inhibition of plasma membrane-based efflux pumps has been observed as well.[8,9] The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic nonconventional antibiotics classifies these compounds as modifiers of antibiotic activity.

In this study Mathan thailam (Traditional Siddha Formulation) and Ethanolic extract Datura are evaluated for their antimicrobial activity. Mathan Thailam, "Pachchai ennai" in Tamil is a popular Siddha formulation used for treatment of burn infection and other skin ailments. The major constituent of it is Datura leaf juice and copper sulphate.

Datura metel Linn *(*Thorn-apple, Devil trumpet, Solanaceae) is the major ingredient of Mathan thailam. Datura is an Indian medicinal plant widely used in phytomedicine to cure diseases such as asthma, cough, convulsion and insanity.[29-31] The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, bronchodilator and hallucinogenic. [29-31] It is used in the treatment of catarrh, diarrhea, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and treachitis.[28] A variety of phytochemicals have been found to occur in *D. metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The solanaceous alkaloids hyoscyamine and scopolamines have been isolated from *D. metel*. Hyoscyamine is the most commonly occurring alkaloid in the solanaceae family and has been associated with varying quantities of hyoscine and in rare cases with traces of atrophine. Ali and Shuaib (1996) isolated a steroidal constituent daturasterol from the leaves of the plant. *D. metel* is an active ingredient in the decoction used presently by herbalists in North Eastern India for the treatment of gonorrhea, asthma, cough, skin ulcers, burns and wounds[29] Several studies have documented the scientific basis for the efficacy of plants in phyto-medicine.

Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome, and the increase in cases of bacterial resistance to aminoglycosides is widely recognized as a serious health threat.[12] In *E. coli,* the main mechanisms of resistance to aminoglycosides are active efflux and enzymatic inactivation.[13] In this work, we tested Mathan thailam and chlorpromazine as a resistance-modifying agent in an aminoglycoside-resistant strain of *E. coli.*

MATERIALS AND METHODS

Strains

The experiments were performed with clinical E. coli isolate (EC27) resistant to neomycin and gentamicin (low level) and to tobramycin, amikacin and kanamycin.[14] The EC-ATCC8539 strain of *E. coli* was used as a positive control. All strains were maintained in Mueller Hinton Agar (Himedia) and MacConkey's agar, and prior to assay, the organisms were grown overnight at 37 ° C in brain heart infusion Agar (Himedia).

Mathan Thailam

The commercially available Mathan thailam was purchased from IMCOPS was used for the experiment. The Method of Preparation of Mathan thailam mentioned in the Formulary of Siddha medicines, the Indian medical practitioner's co-operative pharmacy &stores (IMCOPS), Chennai, 2000, is as follows

- Datura leaf juice 3500 gm
- Coconut oil 1400 gm
- Copper sulphate 175 gm

Dissolve copper sulphate in the juice of Datura leaf and mix oil and heat till dehydrated. Cool and filter.

Plant Material

Leaves of Datura metel were collected from the road sides of Pondicherry. The plant material was identified and a voucher specimen was deposited at the herbarium of Department of Pharmacognosy, Mother Theresa Post Graduate

and Research Institute of Health Sciences (MTPG&RIHS), Pondicherry.

Preparation of EEDM

A quantity of 200 g of dried leaves was dried at room temperature and powdered. The powdered material was extracted by maceration using 1 liter of 95% ethanol as solvent at room temperature, and the homogenate was allowed to stand for 72 h at room temperature. The extracts were then filtered and concentrated under vacuum in a rotary evaporator.[15] For the tests, the dry extract material was dissolved in DMSO.

Drugs

Chlorpromazine, gentamicin, tobramycin, kanamycin, Amikacin and neomycin were obtained from Government Pharmacy, Pondicherry. All drugs were dissolved in sterile water.

Drug Susceptibility Test

The minimum inhibitory concentrations (MIC) of Mathan thailam, antibiotics and chlorpromazine (CPZ) were determined in Mueller Hinton Broth by the micro dilution assay using suspensions of 100000 CFU/ml and a drug concentration range of 1,024–1 µg/ml (2-fold serial dilutions).[16] MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of Mathan thailam as a modulator of antibiotic resistance, MICs of the antibiotics were determined in the presence of Mathan thailam (6.25mg/ml) and CPZ (16µ g/ml) at sub inhibitory concentrations, and the plates were incubated for 24 h at 37 ° C. CPZ was used as positive control for efflux pump inhibition.

RESULTS AND DISCUSSION

The Mathan thailam and EEDM did not show a substantial antibacterial activity at 1,024 µ g/ml against the strains assayed (MIC ≥2,048 µg/ml). However, our experiments with Mathan thailam and EEDM show that they have antibacterial activity against other bacterial species. Although Mathan thailam did not show appreciable antibacterial activity[18], when it was added to the growth medium at 100µl, a dramatic reduction in the MIC for gentamicin was observed in the strain *E. coli* 27 (but not with ATCC8539, possibly due the absence of any resistance mechanisms to gentamicin), demonstrating a potentiating effect of Mathan thailam on aminoglycoside activity (table 1). Mathan thailam with a potentiating effect on gentamicin or other aminoglycosides have not been previously reported. A MIC reduction for kanamycin, tobramycin and amikacin was also observed when CPZ was added to the growth medium at 16 µg/ml (1/4 MIC), which indicates the involvement of an efflux pump in the resistance to these antibiotics (table 1).

Phenothiazines, such as chlorpromazine, probably act on the plasma membrane of bacteria affecting the efflux pumps.[23] This modification of permeability could enhance the activity of antibiotics that act within the cell, such as the aminoglycosides. Efflux pumps have been known as resistance mechanisms of *E. coli* since the 1980s; they are part of the resistance-nodulation-cell division (RND) family of transporters and represent an important mechanism of multidrug resistance that accounts for the resistance to aminoglycosides.[24,25] A potentiating effect of CPZ on gentamicin or neomycin was not observed, which suggests the occurrence of other resistance mechanisms or of a CPZ-insensitive efflux pump that can be blocked by Mathan thailam in the case of gentamicin (table 1).

The results obtained indicate that Mathan thailam could be used as an antibiotic resistance-modifying agent against multiresistant bacteria, as with chlorpromazine.

Table1: MIC values (µg /ml) of aminoglycosides in the absence and presence of Mathan thailam, Ethanolic extract of *Datura metel* (EEDM) and CPZ in *Escherichia coli*

Antibiotic	MIC	EEDM (128µg/ml)	Mathan thailam (6.25mg/ml)	CPZ 16µg/ml
Amikacin	64	64	64	16
Gentamicin	8	8	≤1	8
Kanamycin	256	256	256	8
Neomycin	8	8	8	8
Tobramycin	32	32	32	≤1
Chlorpromazine	64	-	-	-

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