

**AN INVESTIGATION OF
ANTIMICROBIAL COMPOUNDS
FOR IMMUNOMODULATING AND
ANTI-ADHESION PROPERTIES**

**THESIS SUBMITTED
FOR
THE FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY**

BY

NAFISA HASSANALI

**IMMUNOLOGY AND INFECTIOUS DISEASE
RESEARCH LABORATORY
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF KARACHI
2003**

60

27-2-04.

134

PK

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

APPROVAL SHEET

TITLE OF THESIS

An investigation of antimicrobial compounds for immunomodulating and anti-adhesion properties.

NAME OF CANDIDATE

Nafisa Hassanali

THESIS AND SUMMARY APPROVED BY

Thesis Supervisor

(Prof. Dr. Shahana U. Kazmi)

External Examiner

()

Approval Date

THIS PIECE OF MY RESEARCH IS

DEDICATED

TO MY NIECES AND NEPHEWS

ALFEYA, FAZL-E-ABBAS, HUSSAIN,
ABBAS, SHABBIR, ISMAT AND ATOOFA

SO THAT THEY CAN PRODUCE MASTER
PIECES IN THEIR FUTURE LIVES

ACKNOWLEDGEMENTS

I am thankful to Almighty Allah for providing me the strength to complete this dissertation. I appreciate Prof. Dr Shahana U. Kazmi, Chairperson. Department Microbiology, for providing me with an opportunity to re-continue my studies after fifteen years of gap and her patience and tolerance while opening the door of research for a naïve person like me. I feel she has really worked on me to polish my work and personality.

I am indebted to Prof. Dr. Shaheen Faizi (S.I). as she has played a pivotal role in my research career. It was her continuous moral support, guidance and cooperation that led me to achieve this position. I was fortunate enough to avail the facility of not only Prof. Dr. Faizi's Lab, but the library and animal house of Prestigious HEJ Research Institute of Chemistry. It was with the help that I was able to get patent of Amoxy-cassia from the Govt. of Pakistan.

I am thankful to Dr Anne Hook, chairperson Department Microbiology Oxford Ohio, for her keen interest in my work, And introducing me to Dr. Kelly Cowan. I pay my gratitude to Dr. Kelly Cowan, for helping me in the completion of my Ph.D thesis research work. It was under the able guidance of Dr. Kelly Cowan, that I was able to explore medicinal plant with anti adhesive properties.

I am thankful to Chad Asethman, Stephanie and Suzaane, for providing me with a homely atmosphere. and solving all my problems, and making my stay in Oxford, comfortable.

My work would not have been to completion without the help, cooperation and guidance of all members of IIDRL and graduate student of Prof. Dr. Faizi.

At this point of acknowledgement, I do not want to forget my mother. It was her dream, which I have fulfilled with her full support.

I find myself short of words, in thanking Sania Siddiq and Darakhshah, Amber. It is the efforts of Sania and Mariam that this dissertation is in this form.

Nafisa Hassanali

TABLE OF CONTENTS

I. INTRODUCTION / LITERATURE REVIEW	1
A. Infectious diseases and antibiotic resistance.	1
B. Antimicrobial products of plant origin	2
C. Combination therapy for infectious diseases	12
D. Mechanisms of pathogenesis of bacteria	15
i. Cariogenic infections	16
ii. Urinogenital infections	24
E. Effect of plant products on humoral and cell mediated immune responses	26
2. MATERIALS AND METHODS	35
A. Preparation of plant extracts.	35
B. Isolation and identification of bacterial strains.	36
C. Determination of susceptibility of bacterial isolates to standard antibiotics and plant products.	37
i. Agar well diffusion method.	37
ii. Disc diffusion method	37
iii. Minimum inhibitory concentration (MIC) by micro broth dilution method.	38
D. Screening of plant products in combination with antibiotics.	38
i. Synergy by disc diffusion method.	39
ii. Synergy by checkerboard titration.	39
E. Effect of plant products on growth kinetics of susceptible bacteria.	40
F. Effect of plant products on the pathogenesis of cariogenic bacteria.	41
G. Effect of plant products on the attachment of uropathogenic <i>E. coli</i> type 1.	42
H. Flow cytometric evaluation of the effect of plant products on cariogenic and uropathogenic bacteria.	43
I. Effect of plant products on <i>S. agalactiae</i> vaginal infections in rats.	45
J. Toxicity of plant products for BALB/c mice.	46
K. Effect of plant products on host immune response.	46
i. Anti- SRBC antibody titer by haemagglutination (HA).	47
ii. Hemolytic plaque assay for detecting anti-SRBC antibody secreting plasma cells.	48
3. RESULTS	50
A. Isolation and identification of bacterial strains and their susceptibilities against standard antibiotics.	50
B. Antimicrobial activity of plant products.	50
C. Synergistic activity of Amoxy-cassia.	52
D. Effect of plant products on growth kinetics of bacteria.	54
E. Effect of plant products on the pathogenesis of cariogenic bacteria.	55
F. Effect of plant products on uropathogenic <i>E. coli</i> type 1.	58

G. Flow cytometric evaluation of anti-adhesion properties of plant products.	59
H. Effect of plant products on <i>S. agalactiae</i> vaginal infections in rats.	59
I. Toxicity of plant products for BALB/c mice.	60
J. Immunomodulation of host response by plant products.	61
4. DISCUSSION	63
A. Antimicrobial properties of plant products.	65
B. Amoxy-cassia: a new synergistic formulation for MDR <i>Salmonella</i> and MRSA.	70
C. Immunomodulating properties of Amoxy-cassia.	74
D. Anti-infective plant products for cariogenic and uropathogenic bacteria.	76
5. CONCLUSIONS	91
6. REFERENCES	92

LIST OF TABLES

1. LIST OF MEDICINAL PLANTS USED.
2. LIST OF BACTERIAL ISOLATES USED.
3. LIST OF STANDARD ANTIBIOTICS USED.
4. IDENTIFICATION SCHEME FOR GRAM NEGATIVE RODS
5. IDENTIFICATION SCHEME FOR GRAM POSITIVE RODS
6. IDENTIFICATION SCHEME FOR GRAM POSITIVE COCCI
7. ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES AGAINST STANDARD ANTIBIOTICS.
8. MINIMUM INHIBITORY CONCENTRATION (MIC) OF STANDARD ANTIBIOTICS AGAINST BACTERIAL ISOLATES
9. ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES AGAINST MEDICINAL PLANT EXTRACTS.
10. MINIMUM INHIBITORY CONCENTRATION (MIC) OF MEDICINAL PLANT EXTRACTS FOR BACTERIAL ISOLATES
11. MINIMUM INHIBITORY CONCENTRATION (MIC) OF DIFFERENT FRACTIONS OF *C. FISTULA* AGAINST MULTIDRUG RESISTANT *SALMONELLA TYPHI*.
12. MINIMUM INHIBITORY CONCENTRATION (MIC) OF SIAMIMIN (PURE COMPOUND OBTAINED FROM *BOMBAX CEIBA*) FOR BACTERIAL ISOLATES.
13. SYNERGISTIC ACTIVITY OF AQUEOUS EXTRACTS OF DIFFERENT PARTS OF *C. FISTULA* WITH AMOXICILLIN BY AGAR WELL DIFFUSION METHOD.
14. RESULT OF CHECKER BOARD SYNERGY TESTING OF AQUEOUS EXTRACT OF FRUIT OF *C.FISTULA* IN COMBINATION WITH AMOXICILLIN AGAINST MDR *SALMONELLA TYPHI*.
15. RESULT OF CHECKER BOARD SYNERGY TESTING OF *CASSIA FISTULA*, AMOXYCASSIA AND AMOXYCILLIN FOR *SALMONELLA TYPHI*.

16. RESULT OF CHECKER BOARD TITRATION OF *C. FISTULA* (AQUEOUS FRUIT EXTRACT) AMOXICILLIN ALONE AND IN COMBINATION FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*.
17. RESULT OF CHECKER BOARD SYNERGY TESTING OF *CASSIA FISTULA*, AMOXYCASSIA AND AMOXICILLIN FOR *E. COLI*
18. RESULTS OF CHECKER BOARD SYNERGY TESTING OF SHEMAMIN AND AMOXICILLIN AGAINST *SALMONELLA TYPHII*
19. HEMAGGLUTINATION TITER IN MICE TREATED WITH DIFFERENT ANTIMICROBIAL SUBSTANCES
20. ANTI-SRBC SECRETING SPLEEN CELLS IN BALB/c MICE TREATED WITH DIFFERENT ANTIMICROBIAL SUBSTANCES.

LIST OF FIGURES

1. STRUCTURES OF COMMON ANTIMICROBIAL PLANT CHEMICALS.
2. TIME KILL CURVE OF MDR *SALMONELLA TYPHI* IN PRESENCE OF AMOXY-CASSIA, *CASSIA FISTULA* AND AMOXICILLIN.
3. TIME KILL KINETICS OF MDR *SALMONELLA TYPHI* IN PRESENCE OF PE2 (FRACTION OF AMOXY-CASSIA) WITH AND WITHOUT AMOXICILLIN.
4. TIME KILL CURVE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN PRESENCE OF AMOXY-CASSIA, *CASSIA FISTULA* AND AMOXICILLIN.
5. ENHANCED EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVOCADO) ON THE ADHERENCE OF *S. SANGUIS* TO HUMAN 'O' RBC.
6. DECREASED EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVOCADO) ON THE ADHERENCE OF *S. SANGUIS* TO HUMAN 'O' RBC.
7. EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT) ON THE ADHERENCE *S. SANGUIS* TO HUMAN 'O' RBC.
8. EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *SOLANUM TUBEROSUM* (POTATO) ON THE ADHERENCE OF *S. SANGUIS* TO HUMAN 'O' RBC.
9. ENHANCED AGGREGATION OF *S. SOBRINUS* WITH HIGH MOLECULAR WEIGHT DEXTRANE IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVACADO).
10. DECREASED AGGREGATION OF *S. SOBRINUS* WITH HIGH MOLECULAR WEIGHT DEXTRANE IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVACADO).
11. ENHANCED AGGREGATION OF *S. SOBRINUS* WITH HIGH MOLECULAR WEIGHT DEXTRANE IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT).
12. DECREASED AGGREGATION OF *S. SOBRINUS* WITH HIGH MOLECULAR WEIGHT DEXTRANE IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT).

13. AGGREGATION OF *S.SOBRIANUS* WITH HIGH MOLECULAR WEIGHT DEXTRAN IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PORPHYRA TENERA*.
14. ENHANCED COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVACADO).
15. DECREASED COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVOCADO).
16. ENHANCED COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT).
17. DECREASED COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT).
18. COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *PORPHYRA TENERA* (SEA WEED, NORI).
19. COAGGREGATION OF *A.NAESHILUNDII* WITH *S. SANGUIS* IN PRESENCE OF AQUEOUS AND ORGANIC EXTRACTS OF *SOLANUM TUBEROSUM* (POTATO).
20. EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVOCADO) ON THE HAEMAGGLUTINATION TITER (IIA) OF UROPATHOGENIC *E.COLI* TYPE 1
21. EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT) ON THE HAEMAGGLUTINATION TITER (IIA) OF UROPATHOGENIC *E.COLI* TYPE 1
22. EFFECT OF AQUEOUS AND ORGANIC EXTRACTS OF *PORPHYRA TENERA* (SEA VEGETABLE, NORI) ON THE HAEMAGGLUTINATION TITER (H.A) OF UROPATHOGENIC *E.COLI* TYPE 1
23. FLOW CYTOMETRIC EVALUATION OF HAEMAGGLUTINATION OF *S. SANGUIS* TO HUMAN 'O' GROUP RBC IN PRESENCE AQUEOUS AND ORGANIC EXTRACTS OF *BETA VULGARIS* (BEET ROOT)
24. FLOW CYTOMETRIC EVALUATION OF HAEMAGGLUTINATION OF *S. SANGUIS* TO HUMAN 'O' GROUP RBC IN PRESENCE AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVACADO).

25. FLOW CYTOMETRIC EVALUATION OF AQUEOUS AND ORGANIC EXTRACTS OF *PERSEA AMERICANA MILL* (AVOCADO) ON ADHERENCE OF *E. COLI* TYPE 1 TO GUINEA PIG RBCs.
26. FLOW CYTOMETRIC EVALUATION OF AQUEOUS AND ORGANIC EXTRACTS OF *SOLANUM TUBEROSUM* (POTATO) ON ADHERENCE OF *E. COLI* TYPE 1 TO GUINEA PIG RBCs.
27. LOG OF NUMBER OF *S AGALACTIAE* IN VAGINAL SECRETIONS OF RATS TREATED WITH SALINE.
28. LOG OF NUMBER OF *S AGALACTIAE* IN THE VAGINAL SECRETIONS OF RATS TREATED WITH ASPARAGINASE.
29. LOG OF NUMBER OF *S AGALACTIAE* IN THE VAGINAL SECRETIONS OF THE RATS TREATED WITH PPO

SUMMARY

SUMMARY

Global emergence of resistance in bacteria to the antimicrobial agents has made it difficult for the physicians to find effective therapeutic agents for diseases caused by MDR -TB, MDR - Salmonella, MRSA, *Ps. aeruginosa*, Acinetobacter, Candida etc. Since long infectious disease are being cured by traditional practitioners with the help of herbs and higher plants. At present antibiotics, obtained from molds fungus or synthetically prepared have lost their efficacy. The new trend to combat the pathogens is to use water and organic extracts made from different parts of medicinal plants.

In this study 30 medicinal plants and their different parts were screened for their in-vitro anti- microbial activity, anti-adhesion activity under in -vitro and in- vivo in rat model. their toxicity and immunomodulating properties in BALB/c mice.

Screening for the antimicrobial activity was carried out against twenty different bacterial species that includes ATCC and clinical strains. The screening of antibacterial activity was performed by the agar well diffusion and micro broth dilution method. Beside water solution of *Rosa centrifolia* (rose) and fruit,leaves and stem solutions of *C.fistula* was also included. From among eleven plant spices, only clove aqueous extract showed broad-spectrum of antibacterial activity against *Ps. aeruginosae* and *S. agalactiae* and others. The antibacterial component present in the clove was found to be heat stable as the autoclaving of the solutions did not affect its activity. Aqueous extracts of *Rosa centrifolia* (rose) and *C.fistula* (Amaltas) commonly used as therapeutic agents showed anti-microbial activity *M. lysoditicus* , *Staph. aureus* , *C. pseudodiphtheroid* and *Sh.dysenters* , *S. typhi*, *E. coli* and *S. pyogenes* at significantly higher concentrations . Four fractions of organic extracts i.e. P1=P2, 80MR, EA2 and ALCR from the fruit of *C. fistula* were also were found to inhibit MDR *Salmonella typhi* at a Minimal Inhibitory Concentration (MIC) range of 125 – 500 µg/ml.

Methanolic extract of leaf extract of *Bombax ceiba* was found to be effective against *S. epidermidis*, *S. typhi*, *B. Subtilis*. Shemamin - a novel pure compound isolated from the leaf extract of *Bombax ceiba* showed antimicrobial activity against *Listeria*, *B. subtilis*, *Ps. aeruginosa*, *Sh dysenteri*, *C. pseudo diphtheroid* and *E.cloacae*. Third group of plant products including *Persea americana mill* (avocado) peel, pulp, *Beta vulgaris (beet)* root peel and pulp, *Porphyra tenera* (nori, Sea vegetable) and *Solanum tuberosu* (potato) Peel, sub-peel and core were screened against several pathogens.. All the plant solutions and extracts had MIC value >25mg/ml except for Methanol extract of *Persea americana peel* that had MIC value for *Klebsiella* 6.25mg/ml, *Proteus* 1.5mg/ml, *Candida* 1.5 mg/ml, *Pseudomonas* 1.5mg/ml, *E coli* type 1 12.5mg/ml, *Staph aureus* 6.25mg/ml, *Strep sanguis*, *Strep sobrinus* and *Ecoli* >25mg/ml.

Formulation of synergistic compound

Since it takes a long time to introduce new anti- microbial compound, a short cut strategy is to prepare synergistic formulations . In this study, synergistic preparations were developed by combining fruit solution of *C fistula* that is an important medicine of Ayurvedic and traditional medicine with amoxicillin a broad-spectrum antibiotic that has lost its efficacy. The new formulation developed was named as Amoxy-cassia. It has already been patented by the Government of Pakistan (Patent

serial number 137124). We preferred the use of water solution as both amoxicillin and *C.fistula* fruit have proven efficacy in oral dosage in aqueous form. Amoxy-cassia was formulated first by screening with disc diffusion method and further confirmed by the checkerboard titration method and time kill kinetics.

This formulation is found effective against MDR Salmonella typhi, MRSA and E.coli. MDR S typhi causes Typhoid Fever that is endemic in developing countries like Pakistan whereas MRSA is a common cause of nosocomial infections in the hospitalized patients. It has become the threat to hospital personnel, as it mostly invades the patients with knee and hip implantation. Urinogenital infections are very common especially among the women. Another synergistic combination, which was developed during this study, was between Shamimin and Amoxicillin named as Shamoxcillin. This preparation is found to be effective against *S. typhi* and *E.coli*.

In- vitro anti-adhesion Activity of the Plant Products

Infectious diseases can also be controlled by the inhibition of the attachment of bacterial strains to the host tissue. Because once pathogens are able to attain foothold on to the eukaryotic cell with the help of adhesins present in their pili, they survive there by enveloping themselves with the capsule that is recognized with difficulty by the human immune system. They then produce toxins and harmful products, which damage the tissues or interfere with the metabolic processes.

Most important step in the pathogenesis of the disease is the adhesion of the pathogens to the host surface through the adhesin present in their pili. Periodontal diseases and dental caries are due to the adhesion of bacteria to the salivary pellicle like *S.sanguinis*, Aggregation to the polymer dextran e.g. *S sobrinus* and coaggregation of *A .naeshlundii* to *S sanguinis*. They may enhance subsequent colonization of *Porphyra gingivalis* that is associated with adult periodontitis. Organism was treated with the extracts for one hour. Three models were set up for the study of adhesion. First one was attachment of *S.sanguis* to human 'O' group blood, second was aggregation of *S sobrinus* with dextran and third was coaggregation of *A.naeshlundii* T14VI with *S.sanguis* 35. Importance of controlling the dental plaque lies in the fact that most of the pathogens like Streptococcal species present in the dental plaque is responsible for causing pulmonary disease and bacterial endocarditis. The pathogens invade the lung and heart through oral cavity.

All four plants studied including - *Beta vulgaris* (beet) root, *Persea americana mill* (avocado), *Solanum tuberosum* (potato) and *Porphyra tenera* (sea vegetable -Nori) has the potential of interfering with the adhesion of bacteria to host epithelial surfaces. The most significant feature of this finding is that the water solutions of all the plants are playing an important role in the anti-adhesion activity of these plants. From this we can say that when avocado, Beetroot, Potato and sea vegetable is eaten, the components of the plant material get in contact with the bacteria present in plaque and thereby result in inhibition of colonization of bacteria on the teeth surface. Urinary infections caused mainly by *E.coli* are among the most common infectious diseases. Most of the uropathogenic *E.coli* can express type P. fimbriae and I that contain adhesin, which recognizes cell receptors present on the host cells. In our assays, we found that water extracts of *P. tenera* (sea vegetable) and *B. vulgarts* (beet) root inhibited the adhesion of *E. coli* to guinea pig RBC thus inhibiting infection of oral surfaces. Most significant inhibition of adhesion to RBCs was observation with *P.*

americana (avocado) extract. It would suggest that just like cranberry juice, avocado juice can also be consumed to avoid urinary tract infections with *E. coli* Type - 1. The result of our study reveals that all the four plants studied *Beta vulgaris* (beet) root, *Persea americana mill* (avocado), *Solanum tuberosum* (potato) and *Porphyra tenera* (sea vegetable) has the potential to interfere with the adhesion of all the oral bacteria.

Those combinations in which treated *S. sanguis* and *A. naeshlundii* which showed lower haemagglutination titer as compared to the titer of untreated bacteria were further checked by the flow cytometry technique. The flow cytometry result confirmed our first result obtained. Polyphenol Oxidase (PPO) and asparaginase are the two plants enzymes that show anti-adhesion activity in vitro. These compound were tested for their in-vivo anti-adhesion activity against

S. agalactiae (GBS) that normally resides in the female vagina and cause neonatal meningitis. GBS infects neonates during their passage through the vagina birth canal. It is one of the leading causes of due to neonatal meningitis. From our rat infection model it was concluded that treatment of animals post infection with plant enzymes like polyphenol oxidase, inhibited adhesion of bacteria. Another enzyme - Asparaginase helped in the adhesion of GBS to vagina of the female rats. Thus PPO – plant enzyme can be used instead of antibiotics to avoid GBS infection.

Immune system plays an important role in the control infectious diseases. Enhancement of immune system is another important mode of controlling the infectious disease. In this study of Amoxy cassia and *C. fistula* were found to be effective immunomodulator. In this study effect of Amoxy cassia, fruit solution of *C. fistula* amoxicillin on immune system specific humoral immune system of BALB/c mice employing sheep RBC as the antigen. Uncountable antibodies producing cell were formed in the spleens of the mice treated with Amoxy cassia similarly the haemagglutination titer was highest too *C. fistula* fruit solution also exhibited immune enhancing property. Thus novel synergistic preparation Amoxy cassia has posses dual activity antimicrobial and immune enhancing to combat with the pathogens.. Our newly formed synergistic compound - Amoxy-cassia as well as fruit extract of *C. fistula* exhibited a very significant immunoenhancing effect in BALB/c mice. This was observed as high titer of anti-SRBC antibody as well as uncountable anti-SRBC antibody producing plasma cells in spleens of mice treated with Amoxy-cassia. Control mice treated with saline showed fewer plaques /spleen as compared to mice receiving only *C. fistula* or amoxicillin alone. This study shows that amoxy cassia and *C. fistula* to be an immuno enhancing compound. They have the potential to be an excellent drug for the patient having impaired immune system like AID patients, person suffering from malignancies and immunocompromised host. If we look at the efficacy of the amoxy cassia, it in vitro inhibits the nosocomially acquired MRSA infection.

This is the first report about the immunomodulatory properties of the *C. fistula* fruit extract. Thus the novel synergistic formulation Amoxy-cassia is not only inhibiting the growth of difficult to treat organism including MDR *Salmonella typhi* strains, MRSA and *E. coli* but it also enhances host immune response to specific antigens. Amoxycassia has the potential of being developed into an effective therapeutic agent for treatment of bacterial infections as well as an effective immunoenhancing substance to provide protection against infections.