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Original Article

Study of Antimicrobial Activity of Medicinal Plants Against Various Multiple Drug Resistance Pathogens And Their Molecular Characterization And it's Bioinformatics Analysis Of Antibiotic Gene From Genomic Database With Degenerate Primer Prediction

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Abstract

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens.In the present study, we have selected some medicinal plants to be screened against multi-drug resistant bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and various multiple drug resistant fungi.
***Key words*:** Herbal extracts; Antimicrobial agent; Multi-drug resistant

Introduction

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, over use of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of micro-organisms . The worldwide emergence of multi drug resistant *Escherichia coli* and many other ß-lactamase producers has become a major therapeutic problem [Khan, A.U.et al.2004]. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [Coates, A.et.al, 2002]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains [Kafaru, E, 1994]. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Lewis, K.et al., 2006)

Materials and Methods

Plant materials

Leaves *Hibiscus Rosasinensis, calotropis procera, Stevia rebaudiana, Psidium guajava, Murraya koenigii (curry leaves)*.

Extraction of Plant Materials and their extractive value

Methanol extraction

10 g of air dried powder was placed in 100 ml of methanol in a conical flask and kept in rotary shaker at 150 rpm for 24 h. After 24 h, it was filtered and the solvent was evaporated to make the final volume one-fourth of the original volume. It was stored at 4oC for further studies.

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The extractive values of aqueous and methanolic plant extracts were analysed (Ifeoma *et al*.,2002).

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of the of the plants collected was determined as follows: Phytochemical analysis for major phyto constituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Fadeyi, *et al*., 1989). The plant extracts were screened for the presence of biologically active compounds like glycosides, alkaloids, tannins, flavonoids, saponins and steroids (Hamburger and Hostettmann,1991.)

Microrganisms used

Clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, Bacillus Thuringesis, Pseudomonas aeroginosa. All cultures were bio chemically tested for purity.

Antibiotic resistance of test strains

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion against a number of antibiotics [Islam, B.; Khan et al, 2008]. The potency of antibiotics per disc was as follows, Amoxyclav (Ac), Clindamycin (Cd) (10 µg/ disc each); Clarithromycin (Cw) (15 µg/ disc each); Cefaclor (Cj), Cloxacillin (cx) (30 µg/ disc).

Antimicrobial susceptibility testing

The agar well diffusion method as adopted earlier was used; 0.1 ml of diluted inoculum (105CFU/ml) of test organism was spread on MHA plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl (150 mg/ml) of plant extract and solvent blanks. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

Determination of the minimum inhibitory concentration (MIC)

The methanol extract of plants samples which showed significant antimicrobial activity were selected for determination of MIC. A stock solution of 100 mg/ml was prepared. This was serially diluted to obtain various ranges of concentrations between 5mg/ml to 100mg/ml. 0.5ml of each of the dilutions of different concentrations was transferred into sterile test tube containing 2.0 ml of nutrient broth. To the test tubes, 0.5 ml of test organism previously adjusted to a concentration of 105 cells/ml was then introduced. A set of test tubes containing broth alone were used as control. All the test tubes and control were then incubated at 37oC for 18 h. After the period of incubation, the tube containing the least concentration of extract showing no visible sign of growth was considered as the minimum inhibitory concentration

Prediction of Degenrate Primers:

- Open the website of NCBI.(www.ncbi.nlm.nih.gov)
- Type the name of protein of the sample.
- Accession number will be displayed.
- Open one of the accession numbers.
- Information regarding the protein present in the sample will be opened.
- Open Expasy tools n compute iso-electric point and molecular weight.
- If the molecular weight matches with ours then convert it into FASTA format.
- Copy the FASTA format sequence.
- With help of Expasy tool of reverse translate open Sequence Manipulation Suite (http://www.bioinformatics.org/sms2/rev_trans.html) and do convert protein sequence in to nucleotide.
- Copy the nucleotide sequence and save it.
- Open the page of PRIMER 3 PLUS (http://www.bioinformatics.org/sms2/rev_trans.html) and paste the sequence of nucleotide in given box.
- Click on Pick Primers.
- Save the result page which does give information about left primers, right primers. (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi)
- Repeat the above steps to all plant samples containing protein.

Results and Discussion

Phytochemical Analysis

The Phytochemical screening of the plant extracts revealed the presence of tannins, alkaloids and flavanoids in all the plant extracts studied as shown in Table-1. The presence of saponins, tannins, alkaloids, flavanoids, steroids and cardiac glycosides provoked for an in-depth study on the plant. The metabolites are of

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various pharmacological importances. Many triterpene saponins and their aglycones have been reported to have varied uses as anti-ulcerogenic, anti-inflammatory, fibrinolytic, antipyretic, analgesic and anti-edematous in action. The presence of tannin in most of plant extract could be responsible for possible antitumor and anti oxidant activities (Hostettmann and Marston, 1995).

Table-1: Antibacterial activity of some medicinal plants of clearance

Microbes/Conc.(µg/µl)	*Stevia*	*Guava*	*Curry leaves*	*Hibiscus*
S. aureus	3.5cm	2.2cm	1.9cm	2.0cm
P. aeruginosa	2.1cm	2.0cm	1.8cm	1.5cm
E. Coli	2.2cm	2.5cm	1.6cm	1.4cm
B. thuringisis	2.4cm	2.0cm	1.8cm	1.6cm

Table-2: Zone of inhibition of methanolic plant extract (in cm) *Stevia sebaudiana*

Microbes/conc.(µg/µl)	50	45	30	15	05	03	01	0.5	0.3	0.1
P.aeruginosa	2.3	2.1	1.9	1.7	1.5	1.2	-	-	-	-
B. thuringisis	1.9	1.7	1.5	1.2	1.6	1.4	-	-	-	-
E.coli	2.1	1.8	1.5	1.4	1.3	1.2	-	-	-	-
S.aureus	1.9	1.7	1.5	1.5	1.4	1.3	1.2	-	-	-

Table- 3: Zone of inhibition of methanolic plant extract *Murraya Koenigii*

Microbes/conc.(µg/µl)	15	05	03	01	0.5	0.3	0.1
P.aeruginosa	2.0	1.7	1.2	-	-	-	-
B. thuringisis	1.9	1.7	1.4	1.2	-	-	-
E.coli	2.1	1.9	1.7	1.3	-	-	-
S.aureus	2.0	1.7	1.5	1.4	-	-	-

Table-4: Zone of inhibition of methanolic plant extract of *Psidium guajava*

Microbes/conc.(µg/µl)	03	01	0.5	0.3	0.1
P.aeruginosa	1.4	1.2	-	-	-
B. Thuringisis	2.4	1.5	1.2	-	-
E.Coli	2.1	1.5	1.3	-	-
S.aureus	2.0	1.2	-	-	-

Table-5: Zone of inhibition of methanolic plant extract of *Hibiscus Rosasinensis*

Microbes/conc.(µg/µl)	03	01	0.5	0.3	0.1
P. aeruginosa	2.4	1.5	1.2	-	-
B. thuringisis	2.1	1.3	-	-	-
E. coli	1.9	1.4	-	-	-
S. aureus	2.0	1.2	-	-	-

Table - 6: Observation of Phytochemical test

Samples	*Stevia*	*Curry*	*Guava*	*Hibiscus*
Carbohydrates	-	-	-	-
Glycerin	+	-	-	-
Flavonoids	+	-	+	+
Glycosides	+	+	+	-
Saponin	+	-	+	-
Inulin	+	-	-	-
Tannins	+	-	-	+
Proteins	+	+	+	+
Starch	-	-	-	-
Steroids	+	-	-	-
Triterpenoids	-	-	+	+

Table- 7: Prediction of antibiotic gene using SDS-PAGE

Sample	Dist from well(cm)	Mass in KDA	Mass in DA	Sequence on NCBI
Curry leaves	3.7	16.8	16800	N.A
Guava	3.5	17.3	17300	N.A
Hibiscus	3.6	17.6	17600	Yes
Stevia	3.8	16.8	16800	yes
Marker	2,3.2,3.5,4.5,4.2,4.9	14.3-19.3	14300-193000	-

Conclusion

The ethanolic extracts of *Stevia Rebaudiana, murraya Koenigii,Psidium Guajava and Hibiscus Roasanensis* could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms from community as well as hospital settings. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. We can even modify the variety of our plant sample by the recombinant biotechnology method. The study of bioinformatics we do have obtained degenerate primer which can be further use in DNA sequencing and in PCR reaction of that plant which are not from the copy of DNA. Further online tools can be used for creating database of sequences which are not available on NCBI.

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