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SYNERGISTIC ANTIMICROBIAL ACTIVITY OF EDIBLE HERBS AGAINST MULTI DRUG RESISTANT BACTERIA

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ABSTRACT
The antibacterial activity of the two plant extracts and their synergism were studied against some MDR strains. The tested organisms particularly *Klebsiella pneumoniae* were found to be inhibited by *Murraya koenigii* extract and also the combination of *Murraya koenigii* and *Coriandrum sativum* in comparison to *Coriandrum sativum* alone. The combinations of the two extracts in various ratios were found to be effective towards MDR strains. On increasing the proportion of the *Murraya koenigii* and *Coriandrum sativum* the inhibitory effect increased. The extracts were found to be effective against all the test organisms except *Salmonella typhi*. MIC was determined by using broth dilution method. The results supported the notion that Synergism of extracts in various ratios is inhibitory towards Multi-drug resistant strains.

INTRODUCTION

The development and persistence of multi-drug resistance bacteria has posed increasing challenges to public health. In recent years there has been gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries because plant derived drugs have been reported to be safe and without side effects (Pandey and Rai, 2003). Investigations into the anti microbial mode of action and potential uses of plant volatile oil have regained momentum.

Coriandrum sativum is an annual herb belonging to Apiaceae (Umbellifera) family. It is native to Mediterranean regions and currently cultivated in many countries. Pharmacological studies have shown that coriander has anti-diabetic, hypolipidemic and anti cancer effects (Collins *et al*., 1995). Coriandrum sativum has health supporting reputation. It is used for medicinal purposes such as dyspeptic complaints and loss of appetiteThe oil is fungicidal and bactericidal (Ayfer and Turgay, 2003). *Murraya koenigii* – is a popular leafy-spice used in Asian –Indian cuisine for its authentic flavor and distinct aroma. It is popularly known as curry leaf and has been stimulated since its high anti oxidant and anti carcinogenic potential were reported (Khan *et al*., 1997, Khanum *et al*., 2000). It is a rich source of alpha-tocopheral, beta-caroteine and lutein. An alkaloid Murrayacinine is also found in leaves (Chakraborty *et al*., 1974). Fresh curry leaves have 2.6% essential oils. Sesquiterpenes, b-caryophyline, b-gurjivene. Sesquiterpenes have antibacterial and anti fungal activities (Mahantavya and Balasubramaniam, 2003).

Synergistic interaction between the herbs is vital part of therapeutic efficiency. Synergisms between herbs are used in European traditional medical herbalism oriental systems such as traditional Chinese medicine and Ayurveda generally assume synergy to be taking place (Phillipson, 1999). Synergistic interactions are documented from constituents within a total extract of a single herb as well as between herbs in a formulation (Phillipson, 1999).

In the present investigation two common edible plants *Coriandrum sativum* and *Murraya koenigii* were screened for their antimicrobial and synergistic activity against some of the MDR strains.

MATERIALS AND METHODS

Plant materials

The leaves of *Murraya koenigii* and C*oriandrum sativum* used in this study were collected in and around the regions of Sam Higginbottom Institute of Agriculture technology and sciences. (SHIATS)

Preparation of extract

The leaves of the herbs were air dried and ground to fine powder. The alcoholic extracts of each plant was prepared by adding 20g of powdered material to 100mL of ethanol (70%v/v) in water. The powered material was soaked for 24 h. The extract was filtered by a muslin cloth. The filtrate was taken and evaporated at room temperature. The filtrate after evaporation was diluted with DMSO *i.e.* Dimethyl Sulphoxide.

Microorganism

The bacterial strains were collected from the "Microbial Culture Collection Bank" of SHIATS. The strains were maintained in agar slants. They included: *Shigella dysentriae* (MCCB0042), *Escherichiae coli* (MCCB0038), *Salmonella typhi* (MCCBOO17), *Klebsiella pneumoniae* (MCCB0019) and *Staphylococcus aureus* (MCCB0054).

Antibiotic sensitivity test

The antibiotic sensitivity was performed by using agar disc diffusion method as described by (Bauer *et al.,* 1966). The test culture was swabbed with a sterile swab over the agar surface. The antibiotic discs were placed on agar surface with a help of a sterile forcep and kept for incubation at 37ºC for 24h. The antibiotic susceptibility was assayed by measuring the diameter of the inhibition zone. The inhibition zone was measured in millimeters. The antibiotics used in the study were Penicillin G (10μg), Cephalosporin (30μg) Amoxicillin (10μg), Cephazolin (30μg), Tetracycline (30μg), Azithromycin (15μg), Clarithromycin (15μg) Clindamycin (2μg), Norfloxacin (10μg) and Vancomycin (10μg).

Antimicrobial activity of plant extracts

The agar well diffusion method was used to screen the antimicrobial activity of plant extracts as described by (Collins

T1-Leaf extract of Murraya koeinigii,

T2-Leaf extract of *Coriandrum sativum*

T3-Combination of extract *Murraya koeinigii* and *Coriandrum sativum* in the ratio of 1:1,

T4-Combination of extract of *Murraya koeinigii* and *Coriandrum sativum* in the ratio of 1:2,

T5-Combination of extract of *Murraya koeinigii* and *Coriandrum sativum* in the ratio of 1:3,

T6-Combination of extract of *Murraya koeinigii* and *Coriandrum sativum* in the ratio of 2:1,

T7 Combination of extract of *Murraya koeinigii* and *Coriandrum sativum* in the ratio of 3:1.

Minimum Inhibitory concentration

The minimum inhibitory concentration (MIC) values were determined by broth dilution method (Madigan *et al*., 2003) Varying concentrations of the extracts (1%, 5%, 10%, 15% and 20%) were prepared. Two mL of each concentration was added to each 5mL of nutrient broth containing .2ml of standardized test organism of bacterial cells. The tubes were incubated at 37ºC for 24h and then for 48h. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC.

RESULTS AND DISCUSSION

The antibiotic susceptibility test pattern of organisms are given in Table 1.

As can be seen from Table 1, *Salmonella typhi*, *Shigella dysentriae* are resistant to Cephalosporin (Cp) and *Clarithromycin (*Cw). *Salmonella typhi* was also found to be resistant towards Clindamycin and Vancomycin but *Shigella dysentriae* was sensitive towards *Clindamycin* and *Vancomycin. E.coli* was resistant to Penicillin G, Amoxicilin Clindamycin and Vancomycin. *Klebsiella pneumonia* was resistant to Cephalosporin, Cephazolin, Azithromycin, Clarithromycin and Clindamycin. *Klebsiella pneumoniae* was found to have highest sensitivity towards Vancomycin. The sensitivity of Klebsiella pneumoniae towards Vancomycin (Ayfer and Turgay, 2003). *Staphylococcus aureus* was resistant to Penicillin G, Amoxicillin, Cephazolin, Clarithyromycin, Norfloxacin and Vancomycin was found to show more resistant towards different antibiotics among other microorganisms.

As can be seen from Table 2 the extracts of *Murraya koenigii* and *Coriandrum sativum* and their various ratios did not show any inhibitory effect towards *Salmonella typhi*. Similar results were reported by Jedha *et al*. (2000; Saeed and Tariq, 2007) which showed that *Coriandrum sativum* did not show any antibacterial activity towards *Salmonella typhi*. *Shigella dysentriae* showed inhibitory effect against both the extracts and their ratios. The effect of the individual extracts was less in comparison to the synergism of the extracts. The highest inhibitory effect was found in the ratio of 3:1.

Table 1: Antibiotic susceptibility pattern of test organisms

Test organisms	Diameter of inhibition zone (mm)									
	P-G(10μg)	CP(30μg)	Am(10μg)	Cz(30μg)	T(30μg)	At(15μg)	Cw(15μg)	Cd (2μg)	Nx (10μg)	V(10μg)
Salmonella typhi	26	R	20	16	21	24	14	R	18	R
Shigella dysentriae	R	R	26	16	22	24	R	27	R	18
Escherichiae coli	R	15	R	19	18	25	18	R	24	R
Klebsiella pneumoniae	24	R	12	R	24	R	R	R	25	18
Staphylococcus aureus	R	22	R	R	23	R	24	22	R	R

P-G-Penicillin g, Cp-Cephalosporin, Am-Amoxicillin, Cz-Cephazolin, T-Tetracycline, At-Azithromycin, Cw- Clarithromycin, Cd- Clindamycin, Nx-Norfloxacin, V- Vancomycin

Table 2: The antibacterial activities of ethanolic extracts of *Murraya koenigii, Coriandrum sativum* and synergistic effect of their ratios

Test organisms	Resistant pattern of antibiotics	Senstivity pattern of plant extracts						
		T1	T2	T3	T4	T5	T6	T7
Salmonella typhi	Cp,Cd	0	0	0	0	0	0	0
Shigella dysentriae	PG, Cp Cw,Nx	10	12	13	15	14	14	20
Escherichiae coli	PG,Am, Cd,V	11	0	11	14	15	12	25
Klebsiella pneumoniae	Cp, Cz, At, Cd,Cw	20	11	16	20	25	26	40
Staphylococc aureus	PG,Am, Cz, At, Nx,V	11	11	12	14	13	13	10

R- resistant, Cp-Cephalosporin, Cd- Cindamycin, P-G-Penicillin G, ,Cw- Clarithromycin, Cz-Cephazolin, Nx-Norfloxacin, V- Vancomycin , At-Azithromycin. T1- leaf extract of Murraya koeinigii , T2- leaf exract of Coriandrum sativum,T3-Combination of extract of Murraya koeinigii and Coriandrum sativum in the ratio of 1:1, T4- Combination of extract of Murraya koeinigii and Coriandrum sativum in the ratio of 1:2, T5-Combination of extract of Murraya koenigii and Coriandrum sativum in the ratio of 1:3, T6-Combination of extract of Murraya koenigii and Coriandrum sativum in the ratio of 2:1, T7- Combination of extract of Murraya koenigii and Coriandrum sativum in the ratio of 3:1

Table 3: Minimum inhibitory concentration of the synergism of extracts *Murraya koenigii and Coriandrum sativum* in different ratios

Test organisms	Ratios	Initial MIC (%)	Final MIC (%)
Shigella dysentriae	T7	5	10
Escherichia coli	T7	5	10
Klebsiella pneumoniae	T7	5	5
Staphylococcus aureus	T4	10	10

Initial MIC-24h, Final MIC-48hT4- Combination of extract of Murraya koeinigii and Coriandrum sativum in the ratio of 1:2, T7- Combination of extract of Murraya koenigii and Coriandrum sativum in the ratio of 3:1

Escherichiae coli showed sensitivity towards the effect of *Murraya koenigii* but not against *Coriandrum sativum*. Cantore *et al*. (2004) observed that the essential oil of *Coriandrum sativum* inhibited *E.coli*. The combination of the extract of *Murraya koeinigii* and *Coriandrum sativum* showed increased synergistic effect. Ayfer and Turgay (2003) reported that the alcoholic extracts of *Coriandrum sativum* was not found to show any inhibitory effect on *Klebsiella pneumoniae*. Apart from the individual extracts the synergism of the two extracts was found highly inhibition on *Klebsiella pneumoniae* and showed the highest inhibitory effect among all the tested organisms.

Staphlococcous aureus was sensitive both the extracts. Akerela and Ayinde (1998) observed that the volatile oil of *Murraya koeinigii* showed inhibitory effect towards *Staphylococcous aureus*. *Staphlococcous aureus* was sensitive *Coriandrum sativum* but different results were obtained by Ayfer and Turgay (2003) which reported that *Coriandrum sativum* had no inhibitory effect against *Staphlococcous aureus*. The synergisms of the two extracts were found to be effective in comparison to individual extracts on *Salmonella typhi*.

The minimum inhibitory concentration of the different ratios of the extracts *of Murraya koenigii and Coriandrum sativum* are given in Table 3. Broth dilution method was used to analyse the minimum inhibitory concentration of the test organisms by the extracts in various ratios. The different ratios of extract of *Murraya koenigii and Coriandrum sativum* were found to inhibit the different test organisms. As can be seen from Table 3, Shigella *dysentriae* in the ratio of 3:1 was found to be effective at concentration 5% (initial MIC) and at 10% conc (final MIC). Escherichia coli was found to be sensitive in the ratio 3:1 having initial MIC of 5% and final MIC of 10%. The initial and final MIC of *Klebsiella pneumoniae* was found to be same *i*.e. at 5% concentrations. *Staphylococcus aureus* showed the inhibition in the ratio of 1:2 with initial and final MIC of 10%. In case of different ratios of the extracts which were tested against the organisms the ratio 3:1 was found to be more effective in comparison to other ratios. It showed the highest inhibitory effect towards *Klebsiella pneumonia*.

CONCLUSION

From the present study it can be concluded that the synergism among herbs can be a great reward in the field of Phytodrugs. The synergism among herbs *i*.e. *Murraya koenigii* and *Coriandrum sativum* showed increased antibacterial effect against some of the MDR strains. The tested organisms had shown resistance towards different antibiotics but are sensitive towards the extract. The inhibitory effect increased on the combination of the two extracts. With the increase of multiple drug resistance bacteria the concept of synergism will prove to be helpful in the treatment of various diseases and can be used as raw material for the drugs in comparison to synthetic drugs.

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