INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

IN VITRO ANTIMICROBIAL SYNERGISM OF THREE INDIAN MEDICINAL PLANT EXTRACTS ALONE AND IN COMBINATION WITH DIFFERENT ANTIMICROBIALS AGAINST PATHOGENIC BACTERIAL STRAINS

Sharadadevi D R and Paramjyoti L Swamy *

Department of Biochemistry, Gulbarga University, Kalaburagi-585106, Karnataka, India

*Corresponding Author Email: paramjyothiswamy@gmail.com

Article Received on: 04/12/18 Approved for publication: 14/01/19

DOI: 10.7897/2230-8407.100390

ABSTRACT

Aim: To evaluate the antimicrobial efficacies of independent and various within plant extract combinations of three medicinal plants. The fruits, roots and whole plant of the three medicinal plants viz *Phyllanthus niruri*, *Bergenia ligulata* and *Tribulus terrestris* independently and in combination were comparatively assessed for antimicrobial activity against Gram+ve i.e. *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis (*MTCC 121) and *Enterococcus faecalis* (NCIM 5025) and Gram –ve i.e. *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumonia* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Shigella dysenteriae* (clinical strain) *and Escherichia coli* (MTCC 46). Methods: The interaction between plant extracts and antimicrobial agents was carried out using disc diffusion method and broth dilution methods were used to determine the minimum inhibitory concentration (MIC) of the bacterial strains. Results: The results showed good antibacterial activity against all tested organisms. The efficacies of various extract combinations of each plant sample varied, with the strongest synergistic effect was exhibited by the proportional extract herbal combination of *Phyllanthus niruri*, *Bergenia ligulata* and *Tribulus terrestris* against *E.Coli*. Most combinations demonstrated either a synergistic or indifferent interaction effect against test bacteria with few exhibiting antagonistic effects. The diameter of the zones of inhibition of the extract combinations ranged from 6.0±0.5 to 32.3±0.8. The minimum inhibitory concentration (MIC) of different extracts ranged from 0.78 mg/mL to 25mg/mL. Conclusion: These results indicate better synergistic effect with polyherbal combination rather than a single plant which can enhance the antibacterial potential and hence can be useful in fighting emerging drug resistance microorganisms.

Keywords: *Tribulus terrestris*, *Phyllanthus niruri*, *Bergenia ligulata,* Antimicrobial, Extract combination, Interaction, Synergy.

INTRODUCTION

Today, the ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problem[1]. Thus, there is an urgent need for novel antimicrobials. Nowadays, new antibiotics are not coming into the market those are competent enough to battle with multiple drug resistant pathogenic bacterial strains[2]. Therefore, scientists are continuously trying to develop new drugs. Plant driven natural compounds can provide potential lead for the development of new drug. At present, scientists are investigating to find out antimicrobial synergism within different plant extracts[3].

The concept of synergy on plant extracts and their mechanism of action, interaction with antibiotics and with other medicinal plant extracts plays a vital role in the study of importance of medicinal plants and their effect on different pharmacological activities (synergism)[4]. There are very few reports on synergism and combined action of plant extracts and antibiotics. Sudan for the first time has reported synergism between commercial antibiotics and plant extracts. Many researchers have studied the interaction of antibiotics with plant extracts which may lead to the discovery of novel drugs. Betoni et al. have reported the synergy between 13 antimicrobial drugs and 8 plant extracts against *Staphylococcus aureus* strains.

Synergistic effect from combination of plant extracts or phytochemicals with antibiotics may be new choice for treatment of number of infectious diseases[6].

In present study antibacterial activity of three medicinal plants and their combinations with four antibiotics was carried out. Different pathogenic bacteria possess the greatest medical significance. The organisms *Bacillus subtilis*, *Escherichia. Coli*, *Klebsiella pneumonia* have been reported as causal organism of some infectious disease in human and plants[3]. *Escherichia coli* and *Klebsiella pneumonia* are responsible for various diseases including urinary tract, gastrointestinal tract, wound infections, bacteraemia, pneumonia, septicemia and meningitis[7]. *Staphylococcus aureus* is one of the commonest and most important Gram-positive hospital-acquired organism. It has a high propensity to colonize abnormal skin surfaces and open wounds. *Staphylococcus aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, to life-threatening diseases such as pneumonia, meningitis. *Staphylococcus aureus* remains one of the five most common causes of nosocomial infections, often causing postsurgical wound infections[3,8]. The organisms like *Enterobacter, Salmonella typhi, Enterococcus faecalis* and *Shigella dysenteriae* are implicated to cause severe infections in human, as they are found in multiple environmental habitats[9]. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae.

Tribulus terrestris Linn.,(Puncture Vine, Caltrop, Yellow Vine and Goathead) is a procumbent annual or perennial herb and belongs to Zygophyllaceae family[10]. It is native to southern Europe, Africa, temperate and tropical Asia[11]. *Tribulus terrestris* is adapted to warm, temperate regions and is prevalent in areas

having hot summers and dry soils. In India, *Tribulus terrestris* is found primarily on loose and compact sandy loam soils, and reportedly grows on sand dunes in the desert regions[12]. It is a herbal remedy for various medicinal purposes including the treatment of kidney troubles, particularly stones. A literature survey revealed hypotensive and, cardiac depressant effects and contractile activities on smooth muscles[13]. It has been found to be effective in treating angina pectoris by dilating the coronary arteries and improving the cardiac circulation[14]. *Tribulus terrestris* has been commonly used in folk medicine in Turkey as diuretic and against colicky pains, hypertension and hypercholesterolemia[15]. It has protective effect on genetic damage and stimulates melanocyte proliferation in the treatment of vitiligo[16]. It also exhibits an antibacterial and cytotoxic activity[17]. A nematocidal activity has also been reported by Nandal and Bhatti[18]. It has also shown to reduce the amount of urinary oxalate in rats[19,20.]

Phyllanthus niruri Linn. (Syn. *P. fraternus* Webster), belonging to family Euphorbiaceae, is a common kharif (rainy season) weed found in both cultivated fields and wastelands in India. It is an annual herb with height varying between 30 and 60 cm. Its roots, leaves, fruits, milky juice, and whole plants are used as medicine [21]. Fruits are useful for tubercular ulcers, wounds, sores, scabies and ring worm[22]. The fresh root is believed to be an excellent remedy for jaundice, dropsy and genitourinary infections[23]. The infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses[24,25]. In different parts of India, specially, in Chattisgarh state, it is used as a rich traditional medicine[26]. *Phyllanthus niruri* has shown clinical efficacy in viral Hepatitis B for which no effective specific therapy is available[27]. Fresh juice and powder of dried plant are used most frequently in Ayurvedic preparations [28]. *Phyllanthus niruri* is still widely used in herbal medicine in South America, remaining the most popular remedy for gallstones and kidney stones throughout the continent[29]. In Peruvian herbal medicine, it is also used for hepatitis, urinary infections, and as a diuretic[30]. There are also reports of a host of other activities of different parts of *Phyllanthus niruri* and its constituents[29,31].

Bergenia ligulata Wall belongs to family saxifragaceae. Rhizome is the medicinally used part of this plant which is mostly found in temperate Himalayas from Kashmir to Bhutan and in Khasia hills at 1,500 meter altitude. *Bergenia ligulata* has been shown to possess antidiabetic, diuretic, astringent, cardiotonic, wound healing, antipyretic and anti-hemorrhoidal activities[21,32]. It grows against rocks and is popularly known as Pashanbheda (dissolve the stone), signifying its use in herbal formulations for urolithiasis[33]. The rhizome of *Bergenia ligulata* is one of the major ingredients of Cystones (Himalaya) and Calcury (Charakpharma), the two herbal formulations commonly used for treating kidney stones[34]. In an *in vitro* study, the rhizome of *Bergenia ligulata* is reported to inhibit homogeneous precipitation and growth of calcium phosphate (CaP) and calcium oxalate (CaOx) crystals[35,36]. And *in vivo* (hyperoxaluric rat model) antilithiatic efficacy of its rhizome has been attributed to the combination of antioxidant, diuretic and hypermagnesuric features[37].

In light of the new emerging infectious diseases and the development of resistance in those with existing curatives, one of the strategies employed in traditional herbal medicine to overcome these mechanisms is the combination of herbal remedies. Herbal remedies are often prepared from a combination of several different plant species. The pharmacological effects of such mixtures could be as a result of the total sum of different classes of compounds with diverse mechanisms of action. There have been reports of the total contents of an herbal product showing a significantly better effect than an equivalent dose of a single isolated active ingredient or a single constituent herb[38-39]. These findings suggest that the effects may arise from synergistic mechanisms of herbal ingredients. Synergism occurs when two or more compounds interact in ways that mutually enhance, amplify or potentiate each other's effect more significantly than the simple sum of these ingredients[38]. Present investigation is an effort made to investigate the antimicrobial efficacy of three Indian medicinal plants.

MATERIALS AND METHODS

Chemicals

Nutrient agar was obtained from Hi-Media, Mumbai, India, petroleum ether, chloroform, methanol etc. were obtained from Merck, India

Plant materials

The *Tribulus terrestris L* and *Phyllanthus niruri L* were collected from different regions of Raichur district and authenticated by Department of botany, Gulbarga University Gulbarga, Karnataka India. A vocher specimen (No. HGUG 782, HGUG 198 respectively) is preserved in the herbarium of Dept. of Botany, Gulbarga University. The roots *of Bergenia ligulata* were purchased from Amruth kesari depot Bangalore, Karnataka, India.

Preparation of extracts

Collected plant materials were water cleaned, manually chopped into small pieces and air dried under shade for fifteen to twenty days. After drying, the plant materials were pulverized into fine powder by a grinding in machine and stored in dark airtight container till further use.

Hot extraction method

The ground samples (10g) were sequentially extracted with 25mL/g of petroleum ether, chloroform, methanol and water. The crude extracts were then concentrated invacuo. The concentrated extracts were subsequently dried at room temperature, weighed and used for further experimental studies.

Phytochemical analysis

All the potent extracts were subjected to phytochemical analysis by dissolving them in respective solvents. The extracts were screened for the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, terpenoids, proteins, glycosides, diterpenes and steroids by using the standard procedures[40-43].

Antimicrobial susceptibility test

Microorganisms

The eight disease causing bacterial strains were used for the study in which three were Gram positive -*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis (*MTCC 121)and *Enterococcus faecalis* (NCIM 5025) and five were Gram negative – *Enterobacter aerogenes* (MTCC111), *Klebsiella pneumonia* (MTCC 109), *Salmonella typhimurium* (MTCC98), *Shigella dysenteriae* (clinical strain) *and Escherichia coli* (MTCC 46). All the bacterial strains were procured from Dept. of Biotechnology and Microbiology Gulbarga university, Gulbarga, Karnataka, India. The bacterial strains were grown in the nutrient broth and

maintained on nutrient agar (Hi media Laboratories, India) slants at 4°C.

Antibiotics used

Four antibiotics viz ampicillin (AMP10 mcg/disc), rifampicin (RIF30 mcg/disc), gentamicin (GEN10 mcg/disc) and streptomycin (STR 10 mcg/disc) were used in this study which were purchased from Hi-Media Laboratory Pvt. Ltd.

Standardization of micro organisms

200 µl of overnight cultures of each micro organisms was dispensed into 20 mL of sterilized nutrient broth and incubated at 37°C for 4-6 h to standardize the culture to 10^6 CFU/mL. A loopful of the standard cultures was used for the antimicrobial assay[44].

Screening for antibacterial activity (agar well diffusion assay)

In vitro antibacterial activities of all different extract combinations of *Tribulus terrestris*, *Phyllanthus niruri* and *Bergenia ligulata* were determined by standard agar well diffusion assay[45]. Muller Hinton Agar plates were seeded with 18 h old culture of the isolates. Different extracts were dissolved in 10% (v/v) DMSO in deionized water and made the final concentration of 50mg/mL from this 50 µl of different extract combinations were added into the sterile 6 mm diameter well. 10% (v/v) Dimethyl sulfoxide (DMSO) was used as negative control. A loopful each of the standardized culture of test organisms was streaked on the solidified medium and incubated for 24 h at 37° C. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well using standard (Hi-Media) scale. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The concentrations were determined by the method described by Vollekova *et al.* (2001)[46] with minor modifications. The MIC was determined for the micro-organisms that showed maximum sensitivity to the test extracts. In this method the broth dilution technique was used, where the methanolic extracts of different parts of *Tribulus terrestris, Bergenia ligulata* and *Phyllanthus niruri* was prepared to the highest concentration of 50 mg/mL (stock concentration). By adding sterile distilled water serially diluted (two-fold dilutions) using the nutrient broth and it is later inoculated with 0.2 ml standardized suspension of the test organisms. After 18 h of incubation at 37° C, the test tubes were observed for turbidity. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

RESULTS

The table 1 represents the minimal inhibitory concentrations of three different methanolic plant extracts against bacterial strains. The methanolic extract of *Tribulus terrestris* and *Bergenia ligulata* exhibited significant antibacterial efficacy with low MIC values against *S.aureus* and *Enterobacter* respectively at concentration of 0.78 mg/mL. The least efficacy was shown by *Phyllanthus niruri* extract against *B.Subtilis* and *E.faecalis* which was inhibited at concentration of 25.0 mg/mL. The MIC values for other extracts ranged from 1.563 mg/mL to 12.5 mg/mL.

The data pertaining to the antimicrobial potential of the three plants taken individually with some antibiotics against various bacterial strains is presented in Table 2. The methanolic extract of *Tribulus terrestris* and *Bergenia ligulata* showed maximum zone of inhibition against *E. coli*. *Bergenia ligulata* also exhibited maximum zone of inhibition against *Klebsiella* apart from *E.coli.* However the methanolic extract of *Phyllanthus niruri* exhibited maximum zone of inhibition against *B.subtilis*. In toto all the antibiotics showed antibacterial activity against different bacteria but to a varying level.

Table 3 represents synergistic activity of *Tribulus terrestris* with different standard antibiotics. The synergistic effect was observed against *S.aureus* and *E.coli* when the extract was combined with gentamicin and streptomycin respectively. There have been such reports on synergy in literature like Sibanda and Okoh[47] have reported synergistic effect of acetone extract of *Garcinia kola* seeds and chloramphenicol, amoxicillin and penicillin G. We observed an antagonistic effect in our studies against E.coli when the extract was combined with ampicillin. The remaining combinations of extract with different antibiotics showed indifferent effects. Rakholiya and Chanda[48] have reported an antagonist effect of *T. Cattappa* against *B.Megaterium* and *S.epidermis* with penicillin and against *M.flavus* with ampicillin and ofloxacin.

Table 4 represents synergistic activity of *Phyllanthus niruri* with different standard antibiotics. Here also the synergistic effect was observed against *S.aureus* but the antibiotic used was streptomycin. However an antagonistic effect was observed against *Klebsiella* when it was combined with ampicillin. *Phyllanthus niruri* also exhibited an antagonist effect against *S.typhi* with gentamicin. The remaining combinations of the extract with different antibiotics showed indifferent effects.

Table 5 represents synergistic activity of *Bergenia ligulata* with different standard antibiotics against various bacteria. The synergistic effect was found only against *E.coli,* when the extract was combined with streptomycin. However an antagonistic effect was observed against *Enterobacter* when the extract was combined with rifampacin and against *E.faecalis* when the extract was combined with gentamicin. The remaining combinations of *Bergenia ligulata* with different antibiotics showed indifferent effects.

Table 6 represents the data on the results of the antibacterial activity of the three plant extracts in different combinations. All the three combinations in 1:1 ratio showed better synergistic effect unlike single plant extract. This effect was observed with almost all the antibiotics against all the pathogenic bacteria.

The antibacterial activity of *Tribulus terrestris L* alone (Table 3) and *Phyllanthus niruri* alone (Table 4) had exhibited highest zone of inhibition (26.5±0.8 mm and 25.0±0.5 mm) against *S.aureus* and *E.Coli* respectively and *Phyllanthus niruri* alone (Table 4) had exhibited highest zone of inhibition 25.0±0.57 mm against *S.aureus*. However when the combination of these two plants was used the highest zone of inhibition 30.3±0.3, 29.3±0.3, 27±0.5 and 31.6±0.3 mm was observed against *S.aureus, B.subtilis, E.faecalis* and *S.typhi* respectively.

Table 1: Determination of minimum inhibitory concentration (MIC in mg/mL) of crude extracts of three medicinal plants against standard organisms

Organisms	Concentration (mg/mL)										
	M.E	0.098	0.195	0.39	0.78	1.563	3.125	6.25	12.5	25.0	50.0
S.aureus	T.T	+	+	+	***	–	–	–	–	–	–
	P.N	+	+	+	+	+	+	+	***	–	–
	B.L	+	+	+	+	+	***	–	–	–	–
B.subtilis	T.T	+	+	+	+	+	+	***	–	–	–
	P.N	+	+	+	+	+	+	+	+	***	–
	B.L	+	+	+	+	+	+	***	–	–	–
E.faecalis	T.T	+	+	+	+	+	+	+	+	***	–
	P.N	+	+	+	+	+	+	+	+	***	–
	B.L	+	+	+	+	+	+	+	***	–	–
E.coli	T.T	+	+	+	+	***	–	–	–	–	–
	P.N	+	+	+	+	+	+	***	–	–	–
	B.L	+	+	+	+	***	–	–	–	–	–
S.typhi	T.T	+	+	+	+	+	+	***	–	–	–
	P.N	+	+	+	+	+	+	+	***	–	–
	B.L	+	+	+	+	+	+	+	***	–	–
Shigella	T.T	+	+	+	+	+	+	+	***	–	–
	P.N	+	+	+	+	+	+	***	–	–	–
	B.L	+	+	+	+	+	+	***	–	–	–
Klebsiella	T.T	+	+	+	+	+	***	–	–	–	–
	P.N	+	+	+	+	+	+	+	***	–	–
	B.L	+	+	+	+	***	–	–	–	–	–
Enterobacter	T.T	+	+	+	+	***	–	+	–	–	–
	P.N	+	+	+	+	+	+	***	–	–	–
	B.L	+	+	+	***	+	+	+	–	–	–

T.T; *Tribulus terrestris*, P.N; *Phyllanthus niruri*, B.L;*Bergenia ligulata,* RIF; Rifampicin, STR; Streptomycin,GEN; Gentamicin, AMP; Ampicillin, + indicates presence of turbidity, − indicates turbidity is not observed,*** indicates the MIC value.

Table 2: Antibacterial activity of methanolic extract of three medicinal plants and different antibiotics.

Microorganisms	Zone of inhibition (mm)						
	T.T	P.N	B.L	RIF	STR	GEN	AMP
S.aureus	16.3±0.3	8.3±0.8	13.3±0.8	20.3±0.8	24.0±0.5	18.0±0.5	23.0±0.5
B.subtilis	6.0±0.5	19.0±0.5	8.3±0.33	12.0±0.5	11.0±0.5	20.0±0.5	20.6±0.3
E.faecalis	12.3±0.3	13.6±0.8	14.6±0.3	18.3±0.8	18.0±0.5	19.0±0.57	19.3±0.3
E.coli	20.3±0.5	10.3±0.33	17.0±0.57	21±0.5	21.6±0.3	13.6±0.8	20.0±1.1
S.typhi	14.0±0.5	15.0±0.5	5.6±0.33	17.3±0.6	15.0±0.5	20.3±0.8	18.0±0.5
Shigella	15.0±0.5	12.6±0.3	14.6±0.33	17.6±0.8	19.6±0.6	18.3±0.3	19.0±0.5
Klebsiella	8.0±0.5	18.0±0.5	17.0±0.5	10.6±0.3	20.0±1.1	20.3±0.8	21.6±0.5
Enterobacter	12.0±0.5	12.3±0.3	15.6±0.3	17±0.5	17.6±0.8	21.0±0.5	15.6±0.8

T.T; *Tribulus terrestris*, P.N; *Phyllanthus niruri*, B.L;*Bergenia ligulata,* RIF; Rifampicin, STR; Streptomycin, GEN; Gentamicin, AMP; Ampicillin
* The values are Mean ± SEM (n=3)

Table 3: Synergistic activity of methanolic extract of *Tribulus terrestris* with different antibiotics against bacteria.

Micro-organisms	Zone of inhibition (mm) [Antibiotics + Methanolic extract of [T.T]			
	RIF	STR	Gen	Amp
S.aureus	21.0±0.5	19.0±0.5	**26.5±0.8**	21.6±0.8
B.subtilis	20.0±0.5	23.3±0.3	23.0±0.5	21.0±0.5
E.faecalis	17.3±0.6	20.0±0.5	20±0.5	18.0±0.5
E.coli	24.3±0.3	**25.0±0.5**	24±0.5	16.6±0.3
S.typhi	19.0±0.5	19.0±0.5	20.3±0.8	20.0±0.5
Shigella	18.0±0.5	20.0±0.5	17.0±0.5	19.0±0.5
Klebsiella	19.0±0.5	14.0±0.5	21.0±0.5	19.0±0.5
Enterobacter	20.3±0.3	21.0±0.5	20.3±0.8	22.0±0.5

T.T; *Tribulus terrestris*, RIF; Rifampicin, STR; Streptomycin, GEN; Gentamicin, AMP; Ampicillin
* The values are Mean ± SEM (n=3)

Table 4: Synergistic activity of methanolic extract of *Phyllanthus niruri* with different antibiotics against bacteria

Microorganisms	Zone of inhibition (mm) [Antibiotics + Methanolic extract of [P.N]			
	RIF	STR	GEN	AMP
S.aureus	20.3±1.4	**25.00±0.57**	20.00±0.57	20.00±0.57
B.subtilis	19.6±0.8	20.33±0.88	18.33±0.88	20.66±0.88
E.faecalis	19.66±0.67	19.66±0.33	21.66±0.33	23±0.57
E.coli	20.33±1.20	20±0.57	22±0.57	19±0.57
S.typhi	21.66±0.88	24.33±0.88	14.66±0.88	23±0.57
Shigella	18±0.57	20.33±1.20	19±0.57	18±0.57
Klebsiella	21±0.57	15±0.57	20±0.57	16±0.57
Enterobacter	19±0.57	21±0.57	21±0.57	16±0.57

P.N; *Phyllanthus niruri,* RIF; Rifampicin, STR; Streptomycin, GEN; Gentamicin, AMP; Ampicillin * The values are Mean ± SEM (n=3)

Table 5: Synergistic activity of methanolic extract of *Bergenia ligulata* with different antibiotics against bacteria

Microrganisms	Zone of inhibition (mm) [Antibiotics + Methanolic extract of [B.L]			
	RIF	STR	GEN	AMP
S.aureus	22.0±0.5	20.0±0.5	20.3±0.8	20.6±0.8
B.subtilis	20.6±0.8	20.3±0.88	21.3±0.8	21.0±0.57
E.faecalis	18.0±0.5	21.6±0.33	11.0±0.5	20.0±0.57
E.coli	21.0±0.5	**26.0±0.57**	19.3±0.3	20.6±0.88
S.typhi	23.0±0.5	19.0±0.57	17.0±0.5	20.0±0.57
Shigella	17.0±0.5	20.3±0.88	21.0±0.5	20±0.57
Klebsiella	20.6±0.67	17.6±1.2	20.0±0.5	21±0.57
Enterobacter	13.0±0.5	21.0±0.5	21.0±0.5	21.33±1.2

B.L;*Bergenia ligulata,* RIF;Rifampicin, STR;Streptomycin, GEN; Gentamicin, AMP; Ampicillin *The values are Mean ± SEM (n=3)

Table 6: Synergistic effect of three plant extract in different combinations (T.T + P.N, P.N+B.L, B.L+T.T)

Micro organisms	Zone of inhibition (mm)											
	T.T + P.N				P.N + B.L				B.L+ T.T			
	RIF	STR	Gen	Amp	RIF	STR	Gen	Amp	RIF	STR	Gen	Amp
S.aureus	**30.3 ±0.3**	26.6 ±0.8	25.3 ±0.6	23±0 .5	22.3 ±0.3	25.3 ±0.8	19.3 ±0.6	22 ±0.5	24 ±0.3	**28.6 ±0.8**	21 ±0.6	25 ±0.5
B.subtilis	22.3 ±0.3	24.6 ±0.6	**29.3 ±0.3**	22.6 ±0.3	24.0 ±0.3	22.3 ±0.6	22±0 .3	22.6 ±0.3	**27.3 ±0.3**	24.6 ±0.6	**30 ±0.3**	21 ±0.3
E.faecalis	20.6 ±0.3	21.3 ±0.3	22 ±0.5	**27 ±0.5**	20 ±0.3	23.3 ±0.3	20.3 ±0.5	24.6 ±0.5	20.3 ±0.3	22±0 .33	22.3 ±0.5	25.3 ±0.5
E.coli	22.3 ±0.6	20.6 ±0.8	25.3 ±0.6	21.3 ±0.8	21.3 ±0.6	25.3 ±0.8	**27 ±0.6**	21.6 ±0.8	23.6 ±0.6	**32.3 ±0.8**	24.6 ±0.6	**32.3 ±0.8**
S.typhi	23.6 ±0.3	**31.6 ±0.3**	21 ±1.1	20.3 ±0.3	24.3 ±0.3	24.6 ±0.3	22.3 ±1.1	23 ±0.3	**26.3 ±0.3**	22.3 ±0.3	20.6 ±1.1	20 ±0.3
Shigella	19 ±0.5	22.6 ±0.3	21 ±0.5	19.6 ±0.3	18.3 ±0.5	22.6 ±0.3	21 ±0.5	19.6 ±0.3	20.3 ±0.5	21 ±0.3	23.3 ±0.5	19 ±0.3
Klebsiella	19 ±0.5	20.3 ±0.6	21 ±0.5	23.3 ±0.5	**25.6 ±0.5**	17.6 ±1.1	20.6 ±0.5	23.6 ±0.3	21.3 ±0.5	**26.6 ±0.6**	21.3 ±0.5	20.6 ±0.3
Enterobacter	25.3 ±0.3	22 ±0.5	22.3 ±0.3	23.6 ±0.3	20.3 ±0.3	17.6 ±0.5	21.3 ±0.3	23 ±0.3	22 ±0.3	23.3 ±0.5	23 ±0.3	**30.3 ±0.3**

T.T; *Tribulus terrestris*, P.N; *Phyllanthus niruri*; B.L;*Bergenia ligulata* RIF; Rifampicin, STR; Streptomycin, GEN; Gentamicin, AMP; Ampicillin,*The values are Mean ± SEM (n=3)

Similar synergistic effects were found to be exhibited with combinations of *Phyllanthus niruri L* and *Bergenia ligulata*. The antibacterial activity of *Bergenia ligulata* alone (Table 5) *exhibited* 26.0±0.57 mm zone of inhibition against *E.coli* and *Phyllanthus niruri L* alone (Table 4) had exhibited 25.0±0.57 mm zone of inhibition against *S.aureus.* But when the combination of these two plants was used the highest zone of inhibition 27±0.6 mm against *E.Coli* and 25.6±0.5 mm against *Klebsiella* was observed respectively. Further the highest synergistic effect was observed with the combination of *Bergenia ligulata* and *Tribulus terrestris L*. As already explained in Table 5. *Bergenia ligulata* alone had shown 26.0±0.57 mm zone of inhibition and *Tribulus terrestris L* alone (Table 3) had exhibited (26.5±0.8 mm and 25.0±0.5 mm). However when combination of these two was used the highest zone of inhibition 28.6±0.8 mm was observed against *S.aureus,*, 30±0.3 mm and 27.3±0.3 mm against *B.subtilis,* 32.3±0.8 mm against *E.Coli*, 26.3±0.3 mm against *S.typhi*, 26.6±0.6 mm against *Klebsiella* and 30.3±0.3 mm against *Enterobacter* was observed.

DISCUSSION

Synergistic effects resulting from combinations of antibiotics with various extracts has been studied and experimented by number of studies. In present study all the three combinations i.e *Tribulus terrestris L* and *Phyllanthus niruri L*, *Phyllanthus niruri L* and *Bergenia ligulata* and *Bergenia ligulata* and *Tribulus terrestris L* in 1:1 ratio with antibiotics has showed synergistic activity, but best synergy was observed with combination of *Bergenia ligulata* and *Tribulus terrestris L.* This suggests the potential of these plants to improve the performance of antibiotics evaluated.

Further combination of *Bergenia ligulata* and *Tribulus terrestris L* exhibited synergistic effect with all the four antibiotics against most of the organisms tested. while combination of *Tribulus terrestris L* and *Phyllanthus niruri L*, *Phyllanthus niruri L* and *Bergenia ligulata* exhibited synergy with few antibiotics against some organisms. The synergistic properties of *Bergenia ligulata* and *Tribulus terrestris L* seems to exhibit the synergism against

some pathogenic organisms as compared to other two combinations.

A number of *in vitro* studies have reported the use of plant extracts in combination with antibiotics against some resistant strains[49-51]. Adwan, et al.[52] have reported about *in vitro* interaction between ethanolic extracts of *Rhus coriaria* (seed), *Sacropoterium spinosum* (seed), *Rosa damascene* (flower) and certain known antimicrobial drugs against three multidrug-resistant strains of *Pseudomonas aeruginosa*. The synergy between *R. coriaria* and antibiotics showed a high decrease in MIC and a strong bactericidal activity. These results indicated that combination between *R. coriaria* extract and antibiotics could be useful in fighting emerging drug-resistance *P.aeruginosa*. Similarly Toroglu[53] has reported *in vitro* synergistic effects of different spices and herbs against thirteen microbial species.

Present study has exerted a broad spectrum antimicrobial activity by significantly inhibiting both the Gram-positive and Gram negative bacteria. *E.coli* and *K. pneumonia* are responsible for various diseases including urinary tract, gastrointestinal tract, wound infections, bacteriaemia, pneumonia, septicemia and meningitis[7]. This study signifies the effectiveness of combinatorial effect of these plants against the pathogens by remarkably inhibiting the growth of these bacteria which provides breakthrough potential source for treatment of such disease.

Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug[54]. Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract.

CONCLUSION

Based on the results observed we conclude that extracts in combinations will be more potent as compared to single extract. However the verification of real synergy effects can be achieved through detailed pharmacological investigations and by means of controlled clinical studies performed in comparison with synthetic reference drugs.

ACKNOWLEDGEMENTS

One of the authors Ms. Sharadadevi D R would like to thank University Grants Commission (UGC), New Delhi, India for the financial assistance in the mode of National Fellowship for ST students [No.F117.1/2016-17/NFST-2015-17-ST-KAR-1430 (SA-III/Website) April 2016].

REFERENCES

1. Albuquerque WF, Macrae A, Sousa OV, Vieira GHF, Vieira RHSF. Multiple drug resistant *Staphylococcus aureus* strains isolated from a fish handlers. Brazilian Journal of Microbiology 2007; 38:131-134.
2. Kardar SS. Antibiotic resistance: new approaches to a historical problem. Action Bioscience 2005.
3. Rahman S, Parvez AK, Islam R, Khan MH. Antibacterial activity of natural spices on multiple drug resistant Escherichia coli isolated from drinking water, Bangladesh. Annals of Clinical Microbiology and Antimicrobials 2011; 10: 10.
4. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 2005; 100: 80-84.
5. Betoni JEC, Mantovani RP, Barbosa LN, Stasi LC Di, Fernandes A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Memorias do Instituto Oswaldo Cruz. 2006; 101: 387-390.
6. Ahmed Z, Khan SS, Khan M, Tanveer A, Lone ZA. Synergistic effect of Salvadorapersica extracts, tetracycline and penicillin against Staphylococcus aureus. African Journal Basic Applied Sciences 2009; 2(1-2): 25-29.
7. Ncube B, Finnie JF, Van Staden J. *In vitro* antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. Journal of Ethnopharmacology 2012; 139(1): 81-89.
8. Patel S, Sharma V, Chauhan NS, Dixit VK. An updated review on the parasitic herb of Cuscuta reflexa Roxb. Zhong Xi Yi Jie He Xue Bao 2012; 10(3): 249-255.
9. Maleki S, Seyyednejad SM, Damabi NM, Motamedi H. Antibacterial activity of the fruits of *Iranian torilis leptophylla* against some clinical pathogens. Pakistan Journal of Biological Sciences 2008; 11: 1286-1289.
10. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal plants used in Ayurveda and Sidha. New Delhi: CCRAS, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India. 2000; 3:229.
11. Parker KF. An Illustrated Guide to Arizona Weeds. Tucson (AZ): The University of Arizona Press; 1972 .p.338.
12. Pathak PS. Contributions to the ecology of *Tribulus terrestris Linn*. II. Habitat studies, Agra University Journal of Research Science 1970; 19(2): 149-166.
13. Mossa JS, Al-Yahya MA, Al-Meshal IA, Tariq M. Phytochemical and biological screening of Saudi medical plants. Fitoterapia 1983;54 (4):147–152.
14. Wag B, Ma L, Liu T. 406 cases of angina pectoris in coronary heart disease treated with saponin of *Tribulus terrestris* Zhong Xi Yi Jie He ZaZhi 1990; 10(2): 85–87.
15. Arcasoy HB, Erenmemisoglu A, Tekol Y, Kurucu S, Kartal M. Effect of *Tribulus terrestris* L. saponin mixture on some smooth muscle preparations: a preliminary study. Bollettino Chimico Farmaceutico 1998; 137: 473–475.
16. Lin ZX, Hoult JR, Raman A. Sulphorhodamine B assay for measuring proliferation of a pigmented melanocyte cell line and its application to the evaluation of crude drugs used in the treatment of vitiligo. Journal of Ethnopharmacology 1999; 66(2): 142–150.
17. Ali NA, Julich WD, Kusnick C. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology 2001; 74 (2): 173–179.
18. Nandal SN, Bhatti DS. Preliminary screening of some weeds/shrubsfor their nematicidal activity against Meloidogyne javanica Indian Journal of Nematology 1983; 13: 123-127.
19. Sangeeta D, Sidhu H, Thiud SK, Nath R. Effect of *Tribulus terrestris* on oxalate metabolism in rats. Journal of Ethnopharmacology 1994 ;44(3): 61–66.
20. Anand R, Patnek GK, Kulshreshtha DK, Dawan, BN. Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. Indian Journal of Experimental Biology 1994; 32(8): 548–552.
21. Kirtikar KR, Basu BD. In: Indian Medicinal Plants.3:1935.
22. Agharkar SP. Medicinal Plants of Bombay Presidency. Scientific Publishing Jodhpur, India:1991.p.120–122.
23. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, CSIR, New Delhi: 1956.p.191.
24. Ambasta SP. The useful plants of India. New Delhi: PID, CSIR. 51:1986.

25. Satyavati GV, Gupta AK, Tandon N. Medicinal plants of India. Cambridge Printing Works ICMR, New Delh: 1987; 2.p.166.
26. Caius JF.The Medicinal and Poisonous Plants of India. Scientific Publishers, Jodhpur, India: 1986.p.220–223.
27. Paranjpe P. Indian Medicinal Plants: Forgotten Healers. Chaukhamba Sanskrit Pratisthan, Delhi: 2001.p.48–49.
28. Sastry TCS, Kavathekar KR. Plants for Reclamation of Wastelands. Publication and Information Directorate New Delhi. 1990.
29. Taylor L. Technical Data Report for Chanca Piedra Stone Breaker (*Phyllanthus niruri*). Herbal Secrets of the Rainforest. second ed.2003.p.17–31.
30. Devi MV. Effect of *Phyllanthus niruri* on the diuretic activity of punarnava tablets. Journal of Research and Education in Indian Medicine 1986; 5(1): 11–12.
31. Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. Phytochemicals from *Phyllanthus niruri Linn*. and their pharmacological properties. a review. Journal of Pharmacy and Pharmacology 2006; 58: 1559–1570.
32. Zhang Y, Liao C, Li J, Liu X. A review on resource status, bioactive ingredients, clinical applications and biological progress in *Bergenia*. Journal of Medicinal Plants Research 2011; 5: 4396-4399.
33. Garimella TS, Jolly CI, Narayan S. *In vitro* studies on antilithiatic activity of seeds of *Dolichos biflorus Linn*. and rhizomes of *Bergenia ligulata Wall.* Phytotherapy Research 2001; 15: 351-355.
34. Singh DP, Srivastava SK, Govindarajan R, Rawat AKS. High-performance liquid chromatographic determination of bergenin in different Bergenia species. Acta Chromatographica 2007; 19: 246-252.
35. Joshi VS, Parek BB, Joshi MJ, Vaidya AD. Inhibition of the growth of urinary calcium hydrogen phosphate dihydrate crystals with aqueous extracts of *Tribulus terrestris* and *Bergenia ligulata*. Urological Research. 2005a; 33: 80-86.
36. Joshi VS, Parek BB, Joshi MJ, Vaidya AB. Herbal extracts of *Tribulus terrestris* and *Bergenia ligulata* inhibit growth of calcium oxalate monohydrate crystals *in vitro.* Journal of Crystal growth 2005b; 275: 1403-1408.
37. Bashir S, Gilani AH. Antiurolithic effect of *Bergenia ligulata* rhizome an explanation of the underlying mechanisms. Journal of Ethanopharmacology 2011; 122: 106-116.
38. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine 2001; 8: 401–409.
39. Nahrstedt A, Butterweck V. Lessons learned from herbal medicinal products: the example of St. John's Wort. Journal of Natural Products 2010; 73: 1015-1021.
40. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. second ed. London:Chapman and Hill; 1998.p.54-84.
41. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan. 2000.p.218.
42. Sofowora A. Screening plants for bioactive agents. In:Medicinal plants and traditional medicinal in Africa. 2nd ed. Ibadan: Spectrum Books Ltd; 1993.p.134-56.
43. Trease GE, Evans WC. Pharmacognosy. 15th ed. Berlin:Springer; 2002.
44. Collins CH, Lynes PM, Grange JM. Microbiological Methods. 7th ed. Britain: Butterwort Heinemann Ltd; 1995.p.175-190.
45. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Biologiae et Medecine Experimentalis 1990; 15: 113-115.
46. Vollekova AD, Kostal Ova, Sochorova R. Isoquinoline Alkaloids from *Mahonia aquifolium* stem bark is active against Malassezia sp. Folia Microbiologica 2001; 46: 107-111.
47. Sibanda T, Okoh AI. *In vitro* evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics. African Journal of Biotechnology 2008; 7: 1672-1678.
48. Rakholiya K, Chanda S. *In vitro* interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. Asian Pacific Journal of Tropical Biomedicine 2012; 2: 876–880.
49. Chatterjee SK, Bhattacharjee I, Chandra G. *In vitro* synergistic effect of doxycycline & ofloxacin in combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. The Indian Journal of Medical Research 2009; 130: 475-478.
50. Espina L, Somolinos M, Loran S,Conchello P, Garcia D, Pagan R. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food Control 2011; 22: 896-902.
51. Lowa WL, Martin C, Hill DJ, Kenwarda MA. Antimicrobial efficacy of silver ions in combination with tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. International Journal of Antimicrobial Agents 2011; 37: 162-165.
52. Adwan G, Abu-Shanab B, Adwan K. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. Asian Pacific Journal of Tropical Medicine 2010; 3: 266-269.
53. Toroglu S. In-vitro antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. Journal of Environmental Biology 2011; 32: 23-29.
54. Kamatou GPP, van Zyl RL, van Vuuren SF, Viljoen AM. Chemical composition, leaf trichome types and biological activities of the essential oils of four related salvia species indigenous to Southern Africa. Journal of Essential Oil Research 2006; 18: 72-79.

Cite this article as:

Sharadadevi D R and Paramjyoti L Swamy. *In vitro* antimicrobial synergism of three Indian medicinal plant extracts alone and in combination with different antimicrobials against pathogenic bacterial strains. Int. Res. J. Pharm. 2019;10(3):120-126 http://dx.doi.org/10.7897/2230-8407.100390

Source of support: University Grants Commission (UGC), New Delhi, India, Conflict of interest: None Declared