ANTIBACTERIAL AND SYNERGISTIC STUDIES OF SALSOLA KALI

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ABSTRACT

Salsola kali is an annual herb, found near the Bahawalpur (Cholistan desert) South Punjab, Pakistan. Extract of aerial part of the plant in methanol was tested for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Bacillus subtilis, Streptococcus mutans and Sarcina lutae.* Methanol was found to be best antimicrobial solvent. Minimum Inhibitory Concentration (MIC) of methanolic extract was determined and found to be bactericidal in concentration of 0.5 µg/ml against *Staphylococcus aureus*, *S. pneumoniae, Bacillus subtilis and Streptococcus mutans*. Synergistic antibacterial activity of methanolic extracts was tested with respective solvent extracts of aerial parts of the *Heliotropium strigosum*, *Galium asperuloides* and *Senecio chrysanthemoides* synergistically showed best antibacterial activity against all the bacteria (0.5 µg/ml). The extract of *Salsola kali* and the methanolic extract of *Galium asperuloides* showed best activity against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Streptococcus mutans* (0.5µg/ml). Synergistically *Salsola kali* with *Senecio chrysanthemoides* showed best activity against all the bacterial strains (0.5µg/ml) except *S. lutae* and *S. mutans* . *Salsola kali* with *Heliotropium strigosum* showed best activity against *E. coli, S. pneumoniae, B. subtilis, S. lutae* and *S. mutans* (0.5µg/ml) and inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Key Words: *Salsola kali*, Antibacterial activity, Synergistic activity *Heliotropium strigosum, Galium asperuloides, Senecio chrysanthemoides,*

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INTRODUCTION

According to World Health Organization, (Santose, *et. al.,* 1995) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Elof, *et al.,* 1998).

Klein (1998) has taken view and supported it by strong experimental evidence, that synergism occurs only when the microorganism tend to become drug-fast and is related to inhibition by the second of resistance

microorganism surviving the action of first. Secondly, when drug-fastness does not develop with some facility, synergism commonly does not occur and some degree of antagonism may be observed. The consequence of combining drug is dependent upon the strains of micro-organisms upon the condition under which the organism is subjected to the action of drug.

Salsola kali (Family: Chenopodiaceae) is an annual herb commonly found in Bahawalpur (Cholistan desert) South Punjab, Pakistan. This plant has medicinal importance and is used in traditional medicinal system of their native region by various hakims to fight against disease. This plant is used to investigate the antibacterial activity because of its medicinal properties.

Nascimento, G.F, *et al*., (2000) evaluated the antimicrobial activity of plant extracts and phytochemicals with antibiotic susceptible and resistant microorganisms. The possible synergistic effects when associated with antibiotics were studied with *Achillea millifolium* (yarrow), *Melissa officinalis* (lemon-balm), *Ocium basilucum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzgyum joabolanum* (jambolan) and *Thymus vulgaris* (thyme). The highest antimicrobial potentials were observed for the extracts of *Caryophyllus aromaticus* and *Syzygyum joabolanum*, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic resistance bacteria (83.3%).Association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic resistance bacteria. *Pseudomonas aeruginosa* was inhibited by clove, jambolan, pomegranate and thyme extracts. This inhibition was observed with the individual extracts and when they were used in lower concentrations with ineffective antibiotics.

Soberon J.R. et al., (2007) determined the antibacterial and cytotoxic activities of aqueous and ethanolic extracts of northwestern Argentinian plants used in folk medicine and compared with different commercial antibiotics. *Tripodanthus acutifolius* aqueous extract has lower inhibitory concentrations than cefotaxim against *Acinetobacterfreundii*. *Tripodanthus acutifolius* tincture showed lower MIC and minimal bactericidal concentration (MBC) than cefotaxim for *Pseudomonas aeruginosa*. This extract also showed a MIC/MBC lower than oxacillin for Staphylococcus aureus. The cyto-toxicity of all extracts was compared with that of commercial antibiotics. Rutin (3,3',4',5,7-pentahydroxy flavone 3-beta-rhamnosilglucoside ,iso-quercitrin (3,3',4',5,7-pentahydroxyflavone 3-beta-glucoside) and a terpene would be partially responsible for the antibacterial activity of *T. acutifolius* infusion.

Bonjar*,* et al., (2004) *reported that* forty-five species of 29 plant families used in the traditional medicine by Iranian people, showed antibacterial activities against one or more of the bacterial species: *Bacillus cereus*, *Bacillus pumilus*, *Bordetella bronchiseptica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. No plant showed activity against *Serratia marcescens*, *Bordetella bronchiseptica* being the most susceptible species. All extracts showed the same activity 18 months later.

Camporese, et al., (2003) reported that twenty-one extracts from seven herbal drugs, *Aristolochia trilobata* (Aristolochiaceae) leaves and bark, *Bursera simaruba* (Burseraceae) bark, *Guazuma ulmifolia* (Sterculiaceae) bark, *Hamelia patens* (Rubiaceae) leaves and *Syngonium podophyllum* (Araceae) leaves and bark, used in traditional medicine of Belize (Central America) as deep and superficial wound healers, were evaluated for their anti-bacterial properties. Activity was tested against standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. Almost all the extracts were able to inhibit the growth of one or more of the bacterial strains, except that of *Enterococcus faecalis*.

MATERIAL AND METHODS

Microbial strains of *Staphylococcus aureus* (ATCC 25923) (*S.aureus*). *Escherichia coli* (ATCC 2592) (*E. coli*), *pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Streptococcus pneumoniae* (ATCC 49619) (*S. pneumoniae*) *Bacillus subtilus* (ATCC 6051) (*B. subtilus*) and *Sarcina lutae* ATCC 9341 (*S. lutae*) and *Streptococcus mutans* (ATCC 020572) were obtained from Pediatrics, Microbiology Laboratory, Mayo hospital Lahore.

Plant material

Fresh plants of *Salsola kali* (whole plant (PM # 086) was collected from South Punjab (Bahawalpur Road) on 6th June 2005 and *Senecio chrysanthemoides* (whole plant, PM # 185) and *Galium asperuloides* (PM # 0234) were collected from area between Nathia Gali and Khanuspure , Muree Hill , Pakistan on 2nd October 2005. The plants were identified by Mir Ajab Ali Khan, Professor of Botany Quaid-e-Azam University, Islamabad, Pakistan, Dr. Zaheer-ud-din , Professor of Botany Government College Lahore, Pakistan and voucher specimen were deposit in Prem Madan Herbarium of Lahore College for Women University, Lahore Pakistan.

Salsola kali (whole Plant), *Senecio chrysanthemoides* (whole Plant) *, Galium asperuloides* (whole plant) and Heliotropium strigosum (whole Plant) were air dried, finely gained extracted methanol by soxhelt extraction to yield 15% ,20%, 18% and 25% solvent free extract.

Anti bacterial activity was determined by agar well diffusion method [Norsel and Messley .1977]. This test was performed in triplicate by spreading 12-18 hour old pathogenic bacterial cutters containing approximately 106 - 1010 colony forming unit (CFU/ml) on the surface of nutrient agar plates well (4mm) were dug in the media with the help of sterile metallic borer.

Test samples of different concentrations prepared in Methanol were added (50µl) in their respective wells pure methanol was used as negative control (3mm) other wells were supplemented with reference compounds i.e. Ampicillin, Amoxicillin, Levofloxin, Tetracycline, Vancomycin, Ciprofloxacin and Penicillin as positive control.

Synergistic Activity against bacterial strains was determined by taking equal amount (50µl (1:1))of plant extracts by agar well diffusion method as described before.

RESULTS AND DISCUSSIONS

Seven pathogenic bacterial strains (*Staphylococcus aureus, Escherichia. coli, Bacillus subtilis, Streptococcus pneumoniae , Sarcina lutae, Streptoccus mutans and Pseudomonas aeruginosa*) were used in this study and methanolic extract of *Salsola kali* with three combinations of methanolic extracts of other plants *(Salsola kali + Galium asperuloides, Salsola kali + Senecio chrysanthemoides and Salsola kali + Heliotropium strigosum* with concentrations (250, 100, 50, 10, 5, 1.0, 0.5 µg/ml) were used against each of the seven bacterial strains.

The crude methanolic extract of *Salsola kali* showed highest activity against *S.mutans, S.aureus, B.subtilis* and *S. pneumoniae* while it showed moderate bactericidal activity against *P.aeruginosa*. The crude methanolic extract of *Salsola kali* inhibits the growth of *S.lutae* and E. coli. (**Table-01&05**)

Table – 01 Zone of inhibitions of crude methanol extracts of Salsola kali

Bacterial Strains	**Zone of inhibition in mm**						
	***Concentrations* in µg/ml**						
	0.5	**0.1**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus	21	22	29	32	35	-	-
Escherichia coli	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	18	22	26	20
Streptococcus pneumoniae	20	22	24	-	-	-	-
Bacillus subtilus	17	19	22	-	-	-	-
Sarcina lutae	-	-	-	-	20	18	-
Streptococcus mutans	18	14	13	12	8	-	-

The crude methanolic extract of *Senecio chrysanthemoides* showed highest antibacterial activity against *S.aureus, S.mutans and S.pneumoniae* . The plant extract inhibits the growth of *P.aeruginosa, E.coli ,B.subtilus and S.lutae* **(Table -02&05)**

Table -02 Zone of inhibition of crude methanol extracts of Senecio *chrysanthemoides*

Bacterial Strains	**Zone of inhibition in mm**						
	***Concentrations* in μg/ml**						
	0.5	**1.0**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus,	15	16	18	21	-	-	-
Escherichia coli	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	
Streptococcus pneumoniae	12	14	20	18	21	25	-
Bacillus subtilus	-	-	-	-	-	-	-
Sarcina lutae	-	-	-	-	-	-	-
Streptococcus mutans	23	22	19	18	-	-	-

The methanolic extract of *Galium asperuliodes* showed moderate activity against *S.aureus, S. pneumoniae, S.lutae and E.coli* . While this extract showed good activity by increase the concentration of the extract with *P.aeruginosa* , S.mutans and B.subtilis. **(Table-04 &05)**

Table -04 Zone of inhibition of crude methanol extracts of *Galium asperuliodes*

Bacterial Strains	**Zone of inhibition in mm**						
	***Concentrations* in μg/ml**						
	0.5	**1.0**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus,	-	-	19	26	20	28	
Escherichia coli	-	-	19	12	15	20	-
pseudomonas aeruginosa	-	-	20	28	30	32	-
Streptococcus pneumoniae	-	-	18	16	20	23	-
Bacillus subtilus	-	16	15	18	19	24	26
Sarcina lutae	-	-	-	22	20	20	20
Streptococcus mutans	-	16	13	13	23	34	37

Table -05 MIC value of crude methanol extracts of medicinal plants

Bacterail Strains	MIC (µg/ml)			
	Salsola kali	**Senecio chrysanthemoides**	**Galium asperuloides**	***Heliotropium strigosum***
Staphylococcus aureus,	0.5	0.5	-	0.5
Escherichia coli	-	-	-	0.5
pseudomonas aeruginosa	-	-	-	0.5
Streptococcus pneumoniae	0.5	0.5	-	0.5
Bacillus subtilus	0.5	-	1.0	-
Sarcina lutae	-	-	-	-
Streptococcus mutans	0.5	0.5	1.0	1.0

Synergistic activity: The crude methanolic extract of Salsola kali and *Heliotropium strigosum* showed good activity against *S.lutae, S.pnumiae, E.coli , B.subtilus* and *S.mutans* (0.5µg/ml). The crude methanolic extract of Salsola kali with *Heliotropium strigosum* showed moderate activity against *S.aureus and P.aeruginosa* . **(Table-06&** 09**)**

Table -06 Zone of inhibition of crude methanol extracts of *Salsola kali* and *Heliotropium strigosum*

Bacterial Strains	Zone of inhibition in mm						
	***Concentrations* in µg/ml**						
	0.5	**1.0**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus,	-	35	24	20	19	18	-
Escherichia coli	36	32	30	21	-	-	-
Pseudomonas aeruginosa	-	18	15	16	17	18	-
Streptococcus pneumoniae	23	20	30	27	24	17	16
Bacillus subtilus	19	18	16	15	20	24	-
Sarcina lutae	31	27	16	17	15	14	-
Streptococcus mutans	34	20	18	17	16	-	-

The crude methanolic extract of Salsola kali and Senecio chrysanthemoides showed highest activity against *E.coli, P.aeruginosa B.subtilus, S.aureus* and S.pneumoniae but inhibit the growth of S.lutae and S.mutans (**Table-** 07 & 09**)**

Table -07 Zone of inhibition of crude methanol extracts of *Salsola kali* and Senecio chrysanthemoides

Bacterial Strains	Zone of inhibition in mm						
	Concentrations in µg/ml						
	0.5	**1.0**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus,	35	25	21	20	18	-	-
Escherichia coli	35	32	30	27	24	17	-
pseudomonas aeruginosa	16	17	15	19	27	18	-
Streptococcus pneumoniae	20	22	24	26	20	21	-
Bacillus subtilus	16	16	17	17	17	-	-
Sarcina lutae	-	-	-	-	-	-	-
Streptococcus mutans	-	-	24	19	18	-	-

The methanolic extract of Salsola kali and *Galium asperuloides* showed highest activity against *P.aeruginosa E.coli, Sarcina lutae, Streptococcus mutans, Streptococcus pneumoniae and B.subtilis* . The Plant MIC value ranges from 0.5µg/µl-1.0µµg/l. While this extract showed moderate activity against *S.aerus* .(Table 08 & 09)

Table -08 MIC value of crude methanol extracts of Salsola kali and *Galium asperuloides.*

Bacterail Strains	Zone of inhibition in mm						
	Concentrations in µg/ml						
	0.5	**1.0**	**5.0**	**10**	**50**	***100***	**250**
Staphylococcus aureus,	-	-	34	30	29	24	-
Escherichia coli	41	35	29	27	15	14	
pseudomonas aeruginosa	18	15	16	15	14-	-	-
Streptococcus pneumoniae	-	21	22	28	29	24	-
Bacillus subtilus	37	21	22	28	29	-	-
Sarcina lutae	-	24	30	20	18	15	-
Streptococcus mutans	30	25	21	19	15	-	-

Table -09 MIC value of crude methanol extracts of Salsola kali with other medicinal plants

Bacterial Strains	MIC (µg/ml)		
	Salsola kali + *Heliotropium strigosum*	**Salsola kali + Senecio chrysanthemoides**	**Salsola kali + *Galium asperuloides***
Staphylococcus aureus,	-	0.5	-
Escherichia coli	0.5	0.5	0.5
Pseudomonas aeruginosa	-	0.5	0.5
Streptococcus pneumoniae	0.5	0.5	1.0
Bacillus subtilus	0.5	0.5	0.5
Sarcina lutae	0.5	-	1.0
Streptococcus mutans	0.5		0.5

Table -10 MIC value of different antibiotics (Positive Control)

Bacterial Strains	MIC (µg/ml)						
	Ampicillin	**Amoxicillin**	**Levofloxacin**	**Tetracyclin**	**Vancomycin**	**Ciprofloxacin**	**Penicillin**
S.aureus	10	50	100	30	1.0	5.0	10
E. coli	10	80	150	50	5.0	1.0	20
P.aeruginosa	-	50	-	-	-	5.0	-
S.pneumoniae	-	-	20	20	5.0	5.0	-
B. subtilus	250	50	5.0	-	-	-	-
S.lutae	100	-	10	50	-	10	-
S.mutans	50	30	10	-	1.0	5.0	15

The MIC values of test medicinal plants showed best activity as compared with the different discs of the antibiotics (Ampicillin, Amoxicillin, Levofloxacin, Tetracycline, Vancomycin, Ciprofloxacin and Penicillin) against the test strains of bacteria. (Table -10)

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