

SYNERGISTIC ANTIBACTERIAL STUDIES OF HELIOTROPIUM STERIGOSUM

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ABSTRACT

Heliotropium sterigosum is an annual herb found in south Punjab of Pakistan. Extract of whole plant in methanol was tested for anti bacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Bacillus subtilis*, and *Sarcina lutea*. Minimum Inhibitory Concentration of methanolic extract was determined and found to be bactericidal in concentration of 1000 µg/ml against *streptococcus pneumoniae*, *Escherichia coli*, and *Streptococcus pneumoniae*. Synergistic antibacterial activity of methanolic extracts was tested with respective solvent extracts of seeds of *Withania coagulans*, whole plant of *Hypericum perforatum* and *Pinus roxburgii* (bark). *Staphylococcus aureus* was synergistically inhibited by methanolic extract of *Heliotropium sterigosum* and *Pinus roxburgii* (bark) (1µg/ml) and *Withania coagulans* (1µg/ml). Methanolic extract of *Heliotropium sterigosum* inhibits the growth of *Sarcina lutea* in combination with *Pinus roxburgii* (bark) methanolic extract (0.5µg/ml). Highest synergistic activity was observed by *Heliotropium sterigosum* and *Withania coagulans* methanolic extracts against *Pseudomonas aeruginosa* (0.5µg/ml) and *Bacillus subtilis* (1µg/ml).

Keyword Words: *Heliotropium sterigosum*, antibacterial activity, synergistic activity, *Withania coagulans*, *Pinus roxburgii* and *Hypericum perforatum*.

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INTRODUCTION

The medicinal plants have applications in pharmaceutical, cosmetic, agricultural and food industry. The use of medicinal herbs for curing diseases has been documented in history of all civilizations. Man in the prehistoric era was probably not aware of the health hazards associated with irrational therapy. With advancement in the field of therapeutics, it was concluded that plants contains active principles, which are responsible for curative action of the herbs. Ritch-Krc, et al. reported biological activity of some pinus species by pitch preparations against known human pathogens e.g.; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus fumigatus* [Ritch-Krc,et al.2005]

Antibacterial properties of aqueous and methanolic extracts of 26 medicinal plants used in Mexico to treat gastrointestinal disorders were tested against eight different species of enteropathogens: two *Escherichia coli* species, two *Shigella sonnei* species, two *Shigella flexneri* species, and two *Salmonella* sp. species [Alanis et al., 2005].

Antibacterial activity of Ashwagandha [*Withania somnifera* L. Dunal (Solanaceae; root and leaves)], an Indian traditional medicinal plant has been reported against pathogenic bacteria. Oral administration of the aqueous extracts successfully obliterated salmonella infection in Balb/C mice as revealed by increased survival rate as well as less bacterial load in various vital organs of the treated animals. [Owais, et al., 2005]

The genus *Heliotropium sterigosum*, an annual herb consists of about 10-15 species and belongs to family Boraginaceae. It is widely distributed in the South Punjab specially Sahiwal to Bahwalpure road Pakistan. Few species have also been reported from Lahore. Ethno medicinally the plant is laxative and diuretic. The juice is used as an application to sore eyes, gum boils and sores. The decoction of these plants is used as a cure for pain in the limbs [Kiritikar and Basu 1987]

The alkaloid europine isolated from *Heliotropium bovei* showed both antifungal and insect antifeedant activity [Reina M, et al, 1995]. Individual alkaloids (lasicarpine, heliotrene, supinine and 9-angeloylretronecine N-oxide) were isolated from *H. burseriferum*. [Marquinca G.A, 1989].

Alcoholic extract of *H. indicum* was found to possess dose-dependent anti microbial activity against all the test organism [Rao PR.S Nammi and ADV Raju, 2002].

The increase failure of chemotherapeutics against anti biotic resistant exhibited by pathogenic microbial infectious agent, has led to the screening of several medicinal plants for their potential antimicrobial activity [Martinus, et.al, 2001] [Scazzocchio, et al, 2001].

In search of the antibiotics and protection against infectious disease widespread in Pakistan, *Heliotropium sterigosum* (whole plant) was selected. Its antibacterial activity and synergistic activity with other herbs i.e.; *Pinus roxburgii* (bark), *Withania coagulans* and *Hypericum perforatum* whose antibacterial activity has already reported was checked [Naeem I, and Maimoona A, 2008] [Naeem I, et al, 2007] [Siddique Z, 2008].

MATERIAL METHOD

Microbial strains of *Staphylococcus aureus* (ATCC 25923) *Escherichia coli* (ATCC 2592), *pseudomonas aeruginosa* (ATCC 27853) *Streptococcus pneumoniae* (ATCC 49619) *Bacillus subtilis* (ATCC 6051) and *Sarcina lutea* (ATCC 9341) were obtained from National institute of Health Islamabad.

Plant material

Fresh plants of *Heliotropium sterigosum* (whole plant (PM # 040), *Withania coagulans* (seeds)(PM # 004) were collected from South Punjab (Bahawalpur Road / Sakhi Sarwar) on 6th June 2005 and *Hypericum perforatum* (whole plant, PM # 0131) and that of *Pinus roxburgii* (bark) (PM # 0157) from area between Nathia Gali, Khanaspure and Muree Hill, Pakistan on 2nd October 2005. The plants were identified by Mir Ajab Ali Khan, Professor of Botany Quide-e-Azam university , Islamabad, Pakistan, Dr. Zaheer-ud-din , Professor of Botany Government College Lahore, Pakistan and voucher specimen deposit in Prem Madan Herbarium of Lahore College for Women University, Lahore, Pakistan.

Heliotropium sterigosum (whole Plant), *Withania coagulans* (seeds) *Hypericum perforatum* (whole plant) and *Pinus roxburgii* (bark) were air dried and extract was obtained 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 cultures containing approximately 10⁶ - 10¹⁰ colony forming unit (CFU/ml). On the surface of nutrient agar plates, wells (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.

RESULTS AND DISCUSSION

Six pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Sarcina lutea* and *Pseudomonas aeruginosa*) were used and methanolic extract of one plant (*H.sterigosum*) with three combinations of methanolic extracts of other plants (*H.sterigosum*+*Hypericum Perforatum*, *H.sterigosum*+*Pinus bark (rox)* and *H.sterigosum*+*W.coagulans*) with concentration (1000,250,100,50,10,5 and 1µg/ml.) were used against each of the six bacterial strains.

From the results obtained it was observed that methanol was the best solvent for extracting antimicrobial substances from tested medicinal Plant based on the number of organisms inhibited and the diameter of inhibitory zones produced. It was also observed that different extracts were different in their antimicrobial effectiveness depending on the extractive solvent used. Our results prove favorably the suggestion of Oloke and Kolawole [Oloke and Kolawole, 1988] that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. The crude methanolic extract of *H.sterigosum* showed highest bactericidal activity against *S.aureus*, *P.aeruginosa*, *S. pneumoniae* and *E.coli*. The crude methanolic extract of *H.sterigosum* showed poor antibacterial activity against *B. subtilis* and *S.lutea* (Table 1 and 3)

The *Withania coagulans* seed crude methanolic extract showed good antibacterial activity against *S.aureus*, *B.subtilis* but were moderately active against *E. coli*, *P. aeruginosa*, (Table 1 and 4)

The methanolic extract of *Pinus roxburghii (bark)* showed highest antibacterial activity against the bacterial strain *S.aureus*, *P.aeruginosa* and *E.coli*. Their MIC value ranges from 0.1µg/µl-0.5µg/µl. (Table 1 and 5)

The *Hypericum perforatum* showed highest activity against *S.aureus*, *P. aeruginosa* and *E.coli*. The MIC value of the plant ranges from 0.1µg/µl-0.5µg/l. (Table 1 and 6)

Table -1 MIC value of crude methanol extracts of medicinal plants

Bacterial Strains	MIC ($\mu\text{g/ml}$)			
	<i>Heliotropium sterigosum</i>	<i>Hypericum perforatum</i>	<i>Pinus roxburgii</i> (bark)	<i>Withania coagulans (seed)</i>
<i>Staphylococcus aureus</i>	0.1	0.1	0.1	5.0
<i>Escherichia coli</i>	0.1	0.1	0.1	12.5
<i>Pseudomonas aeruginosa</i>		1.0	0.1	1.0
<i>Streptococcus pneumoniae</i>	5.0	-	-	0.5
<i>Bacillus subtilis</i>	-	0.5	0.5	1.0
<i>Sarcina lutea</i>	-	-	0.1	0.5

Table – 02 MIC value of crude methanol extracts of medicinal plants

Bacterial Strains	MIC ($\mu\text{g/ml}$)			
	<i>Heliotropium sterigosum</i>	<i>Hypericum perforatum</i>	<i>Pinus roxburgii</i> (bark)	<i>Withania coagulans (seed)</i>
<i>Staphylococcus aureus</i>	1500	1500	128	50
<i>Escherichia coli</i>	250	1500	128	20
<i>Pseudomonas aeruginosa</i>	300	150	250	200
<i>Streptococcus pneumoniae</i>	250	300	250	50
<i>Bacillus subtilis</i>	250	200	250	200
<i>Sarcina lutea</i>	260	200	250	250

Table – 03 Zone of inhibition of crude methanol extracts of *Heliotropium sterigosum*

Bacterial Strains	Zone of inhibition in mm						
	1.0 $\mu\text{g/ml}$	5.0 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	250 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$
<i>Staphylococcus aureus</i>	10	11	13	15	20	-	-
<i>Escherichia coli</i>	10	11	12	14	16	20	-
<i>Pseudomonas aeruginosa</i>	10	11	12	14	16	18	-
<i>Streptococcus pneumoniae</i>	18	20	22	24	25	30	38
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-
<i>Sarcina lutea</i>	-	-	-	-	-	-	-

Table – 04 Zone of inhibition of crude methanol extracts of *Withania coagulans*

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	-	27	37	-	-	-	-
<i>Escherichia coli</i>	-	-	15	20	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	10
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	11	14
<i>Bacillus subtilis</i>	-	-	-	-	-	8	-
<i>Sarcina lutea</i>	-	-	-	-	-	15	16

Table – 05 Zone of inhibition of crude methanol extracts of *Pinus roxburgii* (bark)

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	10	11	13	15	17	18	-
<i>Escherichia coli</i>	10	11	12	14	16	18	-
<i>Pseudomonas aeruginosa</i>	10	13	14	17	18	24	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	8	10
<i>Sarcina lutea</i>	-	-	-	-	-	15	16

Table – 06 Zone of inhibition of crude methanol extracts of *Hypericum perforatum*

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	9	11	12	18	19	-	-
<i>Escherichia coli</i>	13	14	15	20	22	23	-
<i>Pseudomonas aeruginosa</i>	10	11	12	14	15	17	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	15	15	-
<i>Bacillus subtilis</i>	-	-	-	-	-	8	10
<i>Sarcina lutea</i>	-	-	-	-	-	15	16

Synergistic activity: As the antibacterial activity of methanol extract was better than all other solvent extracts it was selected to study synergistic effect in combination with respective extract of *Heliotropium sterigosum* with *Hypericum perforatum*, *Withania coagulans*, *Pinus roxburgii* (bark). This combination suggested increase in the inhibitory effect of crude drug extract against different microbes that may or not have developed resistant to many available antibiotics.

Synergistic activity (inhibition is doubled compared to one of the constituent) was observed in as low in amounts as 0.1ug /ml (100ug/ml)methanolic extract of *Heliotropium sterigosum mixed with Withania coagulans*.The growth of *Streptococcus pneumoniae* and *E.coli* was inhibited completely . (Table 7 and 8)

Heliotropium sterigosum also showed synergistic activity with *Pinus roxburgii* (bark) against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Table 7 and 10). The growth of all bacteria was inhibited when methanolic extracts of *Heliotropium sterigosum* and *Hypericum perforatum* were mixed.

The growth of all tested bacterial growth was asserted by mixing the methanolic extracts of *Heliotropium sterigosum* and *Hypericum perforatum*. (Table 7 and 9)

Table – 07 MIC value of crude methanol extracts of *Heliotropium sterigosum* with other (*Pinus roxburgii* (bark) *Withania coagulans*, *Hypericum perforatum*) medicinal plants

Bacterial Strains	MIC (µg/ml)		
	<i>Heliotropium sterigosum</i> + <i>Hypericum perforatum</i>	<i>Heliotropium sterigosum</i> + <i>Pinus roxburgii</i> (bark)	<i>Heliotropium sterigosum</i> + <i>Withania coagulans</i>
<i>Staphylococcus aureus</i>	-	0.1	0.1
<i>Escherichia coli</i>	-	0.1	-
<i>Pseudomonas aeruginosa</i>	-	-	0.1
<i>Streptococcus pneumoniae</i>	-	0.1	-
<i>Bacillus subtilis</i>	-	-	5.0
<i>Sarcina lutea</i>	-	-	-

Table -08 Zone of inhibition of crude methanol extracts of *Heliotropium sterigosum* and *Withania coagulans*

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	39	36	30	22	12	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	30	38	39	40	40	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	-	20	34	36	37	-	-
<i>Sarcina lutea</i>	-	-	-	12	50	-	-

Table – 09 Zone of inhibition of crude methanol extracts of *Heliotropium sterigosum* and *Hypericum perforatum*

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	-	-	-	12	15	20	-
<i>Escherichia coli</i>	-	-	-	10	12	09	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	10	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	17	17	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-
<i>Sarcina lutea</i>	-	-	-	-	-	-	-

Table -10 Zone of inhibition of crude methanol extracts of *Heliotropium sterigosum* and *Pinus roxburgii* (bark)

Bacterial Strains	Zone of inhibition in mm						
	1.0 µg/ml	5.0 µg/ml	10 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
<i>Staphylococcus aureus</i>	34	32	32	28	28	-	-
<i>Escherichia coli</i>	29	27	24	14	16	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	12	-	-	-
<i>Streptococcus pneumoniae</i>	16	14	11	12	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-
<i>Sarcina lutea</i>	-	-	-	12	14	15	-

CONCLUSION

It is concluded that the methanolic extract of *Heliotropium sterigosum* has antibacterial activity and has maximum effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Moreover, it also has synergistic effect when mixed with *Withania coagulans*, *Pinus roxburgii* (bark) and *Hypericum perforatum*.

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