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Impact of *Chenopodium album* and *Allium sativum* extracts alone and in combination against mastitogens

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Noor Ul Absar [1], Rabia Tanvir [1]*, Noreen Sarwar [1] and Qamar Un Nisa[2]

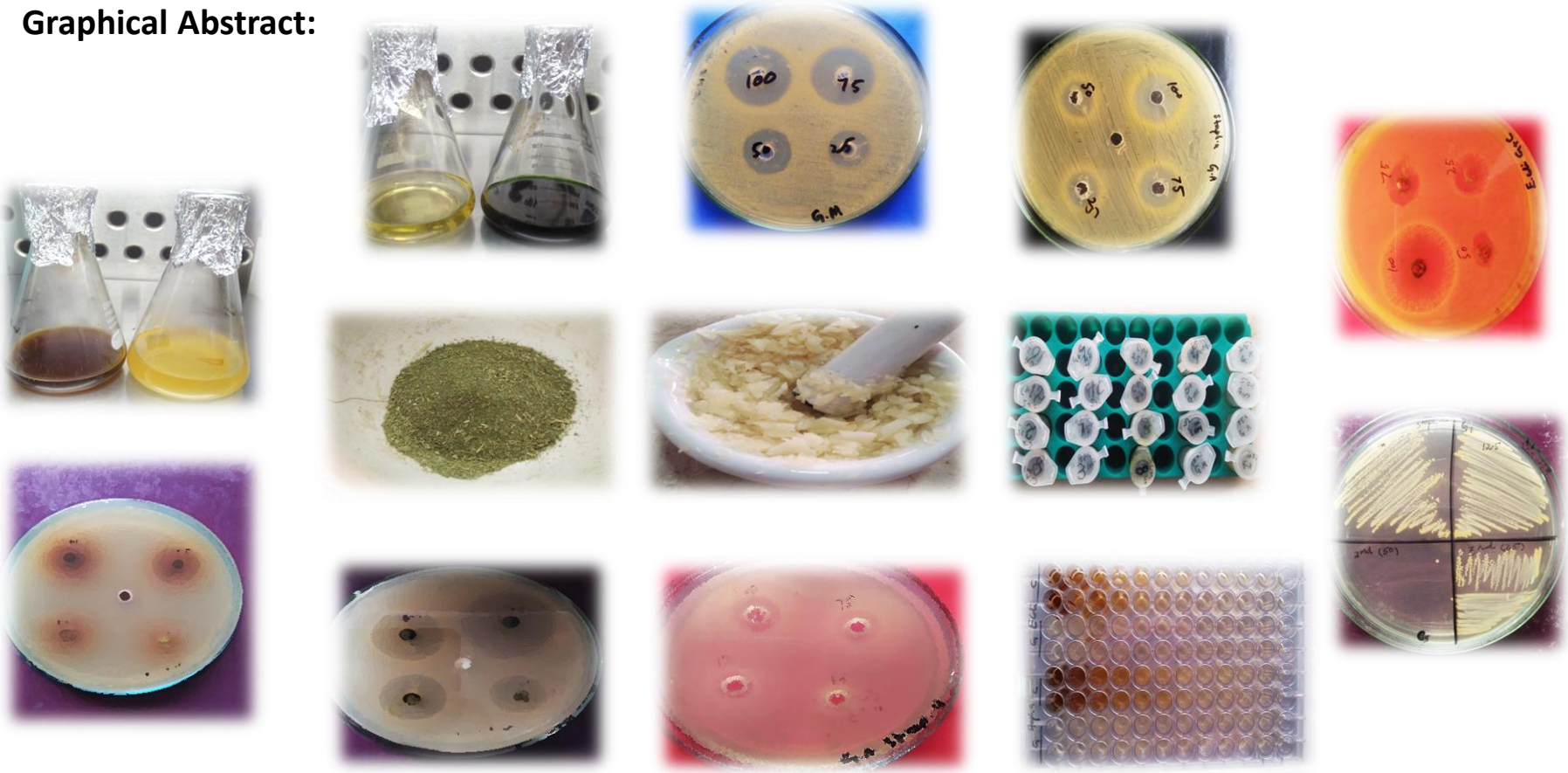
[1]Institute of Microbiology (IOM), University of Veterinary and Animal Sciences (UVAS), Lahore 54000, Punjab, Pakistan
[2]Department of Pathology, University of Veterinary and Animal Sciences (UVAS), Lahore, 54000, Punjab, Pakistan

*Corresponding author: rabia.tanvir@uvas.edu.pk; rabiatanvir@outlook.com

UVAS UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES LAHORE

Impact of *Chenopodium album* and *Allium sativum* Extracts Alone and in combination against mastitogens

Graphical Abstract:



Abstract:

In dairy sector, mastitis is one of the most important and costly disease because due to it the world's economy faces about 35 billion dollar loss each year. Bacterial pathogens that cause mastitis are called mastitogens. Among these bacterial species *Escherichia coli, Staphylococcus aureus, Streptococcus uberis, Streptococcus agalactia* and *Streptococcus dysgalactia* are the most important. Mastitis is treated with antibiotics however; due to their improper, excessive and irrational usage, these pathogens have become resistant to them. Drug residues in milk is also a factor resulting in multi-drug resistance (MDR) in mastitogens that cause treatment to become ineffective. In Pakistan, several ethnoveterinary plants such as *Allium sativum* (Garlic) and *Chenopodium album* (Goosefoot) are used for the treatment of mastitis in cattle and buffalo. For this purpose, we prepared aqueous and methanol extracts of *A. sativum* and *C. album*. In the agar well diffusion method, the aqueous *A. sativum* showed strong activity against *Staphylococcus aureus* i.e. 20mm. whereas, the methanolic extract of *C. album* gave 11mm zone of inhibition against *Staphylococcus aureus.* By combining the extracts of *A. sativum* and *C. album* then they give a synergistic effect especially against *E.coli*. Results showed that zone of inhibition against *Staphylococcus aureus* was 16mm, against *E.coli* 22mm and against *Streptococcus uberis* was 5mm. Our study is in agreement with the use of *A. sativum* and *C. album* in cases of mastitis and recommend their combined use for better results.

Keywords: *Allium sativum*; *Chenopodium album*; Mastitis

Introduction

- *Chenopodium album* (English: Goosefoot, Urdu: Bathoo, Bathua) belongs to the family Chenopodiaceae
- It is a commonly available medicinal plant in Pakistan and its different parts are utilized in the traditional medicines
- It is grown with all other winter crops around the world like in North America, England, Iran and Asia including India and Pakistan
- *C. album* has substances like flavonoid, glucosides, terpenoids and phenolic acid which are responsible for its bioactivity (1)

(1) Khomarlou N, Aberoomand-Azar P, Lashgari AP, Tebyanian H, Hakakian A, Ranjbar R, Ayatollahi SA. 2018. Essential oil composition and *in vitro* antibacterial activity of *Chenopodium album* subsp. *striatum*. Acta Biologica Hungarica. 69(2): 144-155

Introduction (Cont.)

- This plant is used as a treatment for animals
- An anti-helminthic
- Laxative
- To treat gastro intestinal problems (2)

Photo courtesy: Dr. Rabia Tanvir

(2)Chamkhi I, Charfi S, Hachlafi NE, Mechchate H, Guaouguaou F-E, El Omari N, Bakrim S, Balahbib A, Zengin G, Bouyahya A. 2022. Genetic diversity, antimicrobial, nutritional, and phytochemical properties of *Chenopodium album*: A comprehensive review. Food Research International. 110979.

Introduction (Cont.)

- *Allium sativum* (English: Garlic, Urdu: Lehson) is the member of family Alliaceae and belongs to the genus *Allium*
- It is commonly used for the treatment of mastitis in Pakistan (3)
- The chemical compound allicin is considered to play an important role in its activity against gram positive and gram negative bacteria (4)

(3) Ebrahim RA, Gamal RF, Mohamed SH, Abdel-Rahman R. 2018b. Impact of *Allium sativum* against *Enterobacter sp*. as water borne pathogenic bacteria isolated from River Nile. Arab Universities J. Agr. Sci. 26(Special issue (2D): 2525-2531.

(4) Anggraini AL, Dwiyanti RD, Thuraidah A. 2020. Garlic extract (*Allium sativum* L.) effectively inhibits *Staphylococcus aureus* and *Escherichia coli* by *in vitro* test. Tropical Health and Medical Research. 2(2): 61-68.

Introduction (Cont.)

- *A. sativum* is rich in sulfur-containing phytoconstituents such as ajoenes, vinyldithiins, allin, allicin and flavonoids such as quercertin

- Allicin is considered to be the major reason for its antibacterial activity (5)

Cloves of Garlic (*Allium sativum*)
(Photo courtesy Ms. Noor Ul Absar)

(5) El-Saber Batiha G, Magdy Beshbishy A, G. Wasef L, Elewa YH, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. 2020. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. Nutrients. 12(3): 872.

Results and discussion

Characterized bacterial strains of mastitogens (*Staphylococcus aureus, Escherichia coli, Streptococcus uberis)* were revived to check the plant extracts activity against them

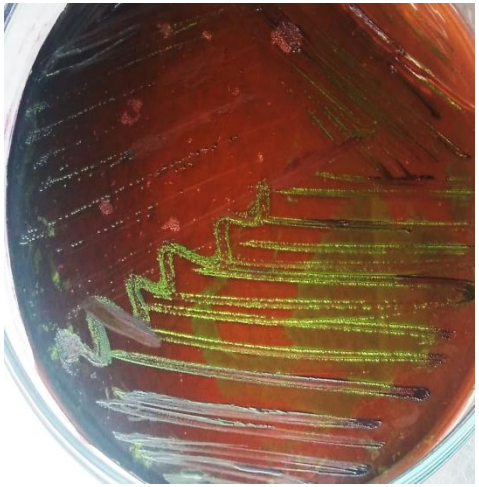


Photo courtesy : Ms. Noor Ul Absar

Results and discussion (Cont.)

Antibiotic profiling for mastitogens was done using antibiotic susceptibility testing (AST) following CLSI guidelines. The zones of inhibition was measured in mm (s)

1. Results against *S. aureus*

Antibiotic Disc (ug)	*Zone of Inhibition (ZOI) (mm)
Vancomycin 20ug	I (20mm)
Erythromycin 15ug	S (24mm)
Clindamycin 2ug	S (36mm)
Ciprofloxacin 5ug	S (33mm)
Trimethoprim 25ug	R (14mm)

S= Susceptible, I= Intermediate, R= Resistant; *CLSI 2020

Results and discussion (Cont.)

2. Results against *E. coli*

Antibiotic Disc (ug)	*Zone of Inhibition (ZOI) (mm)
Clindamycin 2ug	R (no zone)
Amikacin 30ug	R (19mm)
Trimethoprim 25ug	R (20mm)
Ciprofloxacin 5ug	S (33mm)
Lincomycin 10ug	R (no zone)
Vancomycin 30ug	R (no zone)

S= Susceptible, I= Intermediate, R= Resistant; *CLSI 2020

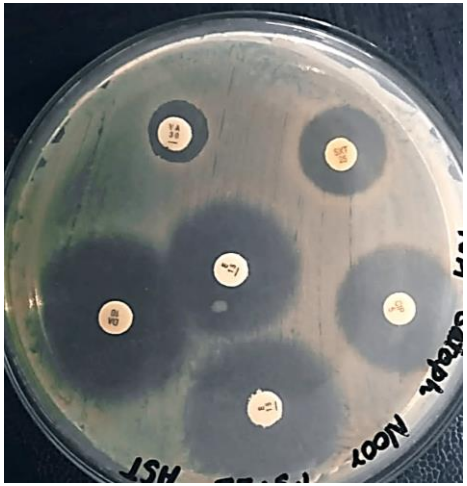
Results and discussion (Cont.)

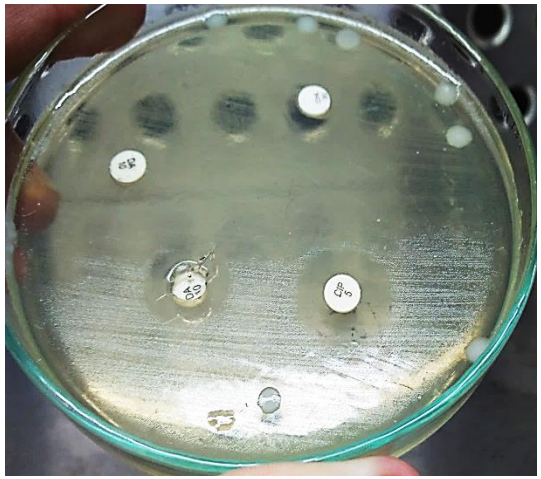
3. Results against *S. uberis*

Antibiotic Disc (ug)	*Zone of Inhibition (ZOI) (mm)
Erythromycin 15ug	R (13mm)
Lincomycin 10ug	R (5mm)
Vancomycin 30ug	R (11mm)
Clindamycin 2ug	R (14mm)
Chloramphenicol 20ug	S (26mm)
Ciprofloxacin 5ug	R (15mm)

S= Susceptible, I= Intermediate, R= Resistant; *CLSI 2020

Results and discussion (Cont.)





Zone of inhibition displaying the antibiotic susceptibility pattern for *S. aureus*, *E. coli* and *S. uberis* following CLSI guidelines (Photo courtesy : Ms. Noor Ul Absar)

Results and discussion (Cont.)

Preparation of aqueous and methanol extracts of *C. album* and *A. sativum* (20g/100ml)





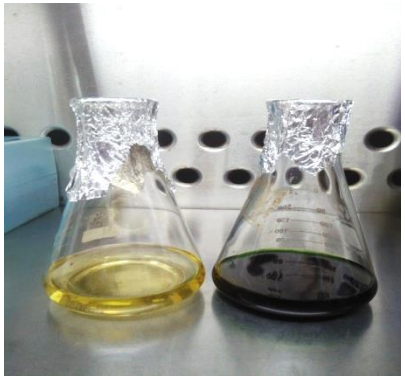



Photo courtesy : Ms. Noor Ul Absar

Results and discussion (Cont.)

Percentage yield % of plant extracts

Plant name	Extract (s)	Quantity of solvent (ml)	Weight (gm) of plant powder or crushed cloves (Garlic)	Extract weight (gm)	Percentage yield %
A. sativum (Garlic)	Methanol	100	20	4.54	22.74
	Distilled water	100	20	6.69	33.75
C. album (Bathoo)	Methanol	100	20	1.789	8.945
	Distilled water	100	20	3.61	18.05

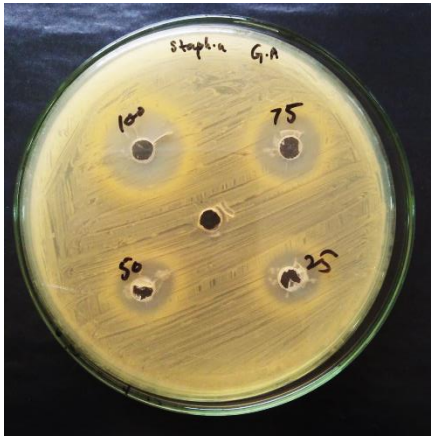
Results and discussion (Cont.)

1. Antibacterial activity of aqueous extracts of *A. sativum* against *S. aureus, E. coli* and *S. uberis* at concentration of 25%, 50%, 75% and 100%

Mastitogen	25% ZOI (mm)	50% ZOI (mm)	75% ZOI (mm)	100% ZOI (mm)
Staphylococcus aureus	10	12	13	15
	12	13	18	20
	25	30	35	40
	M=16	M=18	M=22	M=25
E.coli	14	15	22	24
	11	13	14	17
	9	10	12	15
	M=11	M=13	M=16	M=19
Streptococcus uberis	7	8	10	11
	0	0	0	0
	0	8	9	11
	M=2	M=5	M=6	M=7

ZOI= Zone of inhibition; M= Mean value; (-) = no zone of inhibition

Results and discussion (Cont.)



Antibacterial activity of aqueous extracts of *A. sativum* against *S. aureus, E. coli* and *S. uberis* at 25%, 50%, 75% and 100% concentration. A control is indicated that contained distilled water (Photo courtesy : Ms. Noor Ul Absar)

Results and discussion (Cont.)

2. Antibacterial activity of methanol extracts of *A. sativum* against *S. aureus, E .coli* and *S. uberis*

Mastitogen	25% ZOI (mm)	50% ZOI (mm)	75% ZOI (mm)	100% ZOI (mm)
Staphylococcus aureus	15	17	22	23
	15	17	23	24
	11	16	19	20
	M=14	M=17	M=21	M=22
E.coli	-	11	20	30
	-	10	12	13
	-	9	10	12
	-	M=10	M=14	M=18
Streptococcus uberis	-	-	19	19
	-	-	12	15
	-	-	15	16
	-	-	M=15	M=17

ZOI= Zone of inhibition; M= Mean value; (-) = no zone of inhibition

Results and discussion (Cont.)

Antibacterial activity of methanolic extracts of *A. sativum* against *S. aureus, E. coli* and *S. uberis* at 25%, 50%, 75% and 100% concentration (Photo courtesy : Ms. Noor Ul Absar)

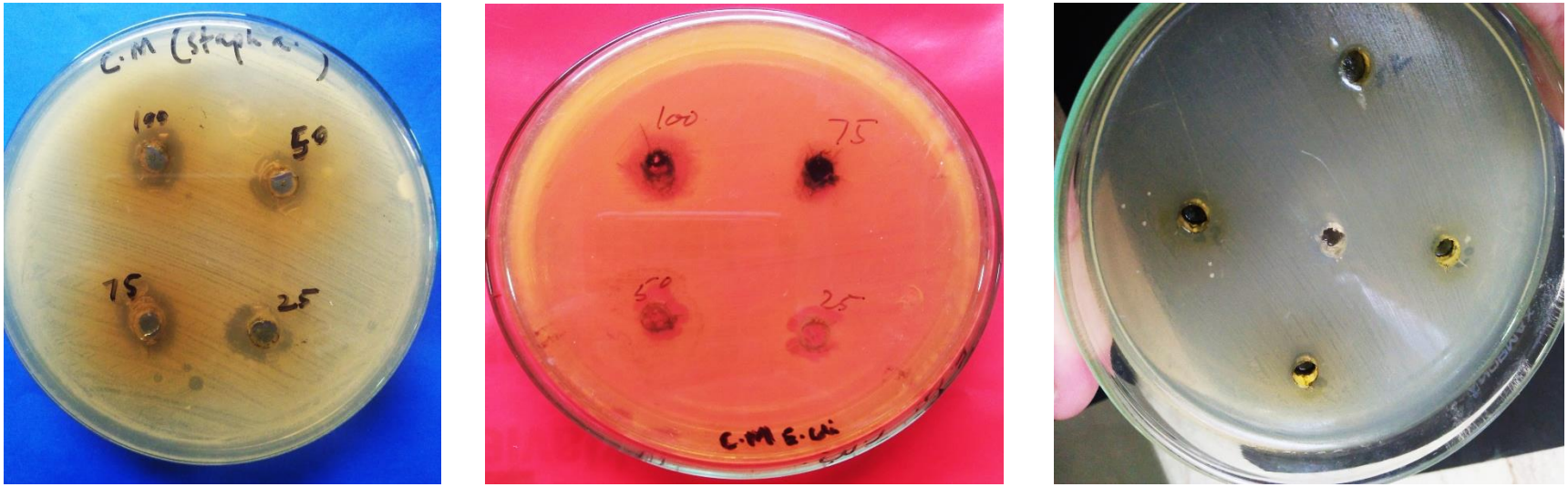
Results and discussion (Cont.)

3. Antibacterial activity of methanol extract *of C. album* against *S. aureus, E.coli* and *S. uberis*

Mastitogen	25% ZOI (mm)	50% ZOI (mm)	75% ZOI (mm)	100% ZOI (mm)
Staphylococcus aureus	11	12	12	13
	6	8	10	11
	8	13	15	20
	M=8	M=11	M=12	M=15
E.coli	-	-	-	15
	-	-	-	10
	-	-	-	9
	-	-	-	M=11
Streptococcus uberis	8	10	10	12
	-	6	7	10
	-	7	10	13
	M=3	M=8	M=9	M=12

ZOI= Zone of inhibition; M= Mean value; (-) = no zone of inhibition

Results and discussion (Cont.)



Antibacterial activity of methanolic extract *of C. album* against *S. aureus, E. coli* and *S. uberis* at 25%, 50%, 75% and 100% concentration (Photo courtesy : Ms. Noor Ul Absar)

Results and discussion (Cont.)

4. Aqueous extract of *C. album* show antibacterial activity only against *E.coli*

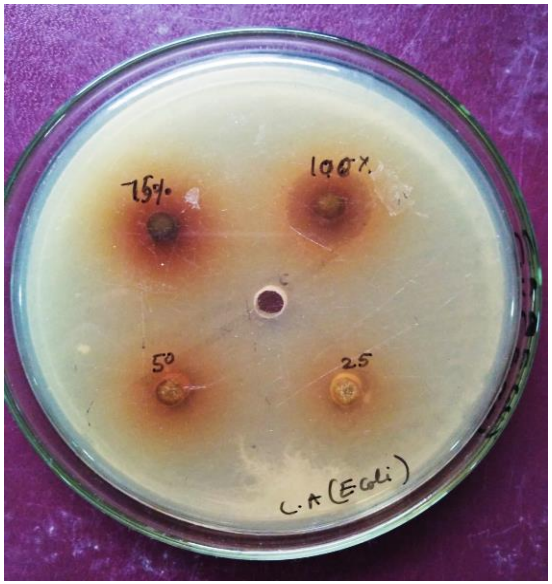


Photo courtesy : Ms. Noor Ul Absar

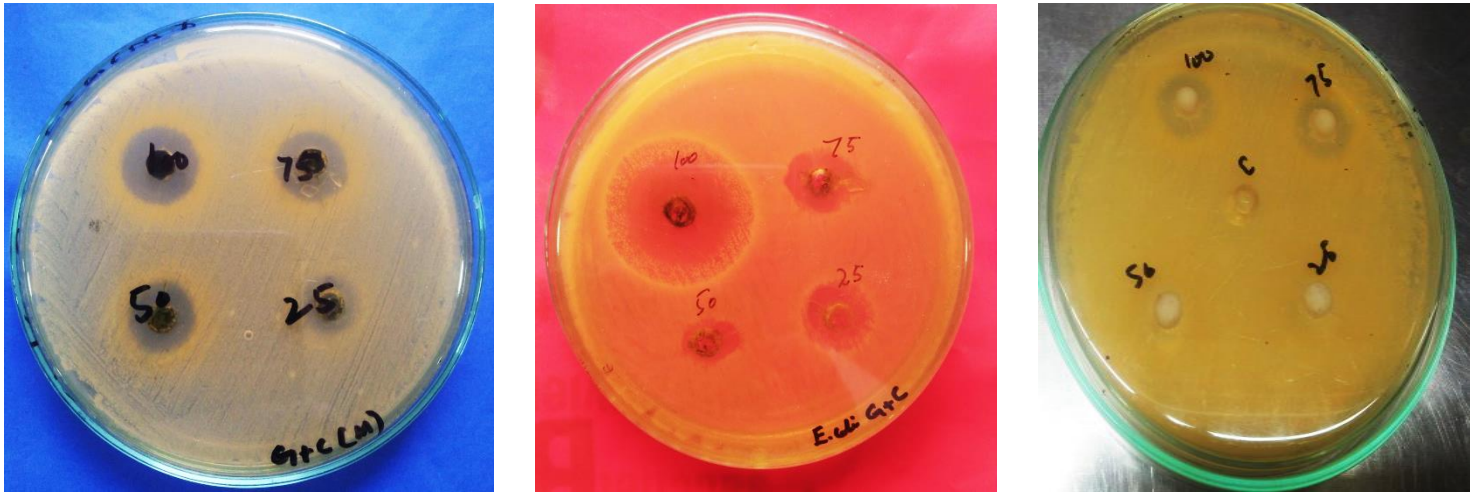
Results and discussion (Cont.)

5. Antibacterial activity of combination of both plant extracts against *S. aureus, E.coli* and *S. uberis*

Mastitogen	25% ZOI (mm)	50% ZOI (mm)	75% ZOI (mm)	100% ZOI (mm)
Staphylococcus aureus	11	13	14	16
	11	16	19	20
	10	15	20	23
	M=11	M=15	M=18	M=20
E.coli	15	13	14	25
	19	23	25	35
	17	23	24	33
	M=17	M=20	M=21	M=31
Streptococcus uberis	-	6	8	15
	-	6	7	8
	-	-	-	20
	M=0	M=4	M=5	M=14

ZOI= Zone of inhibition; M= Mean value; (-) = no zone of inhibition

Results and discussion (Cont.)



Antibacterial activity of combined methanolic extracts *of A. sativum and C. album* against *S. aureus, E. coli* and *S. uberis* at 25%, 50%, 75% and 100% concentration (Photo courtesy : Ms. Noor Ul Absar)

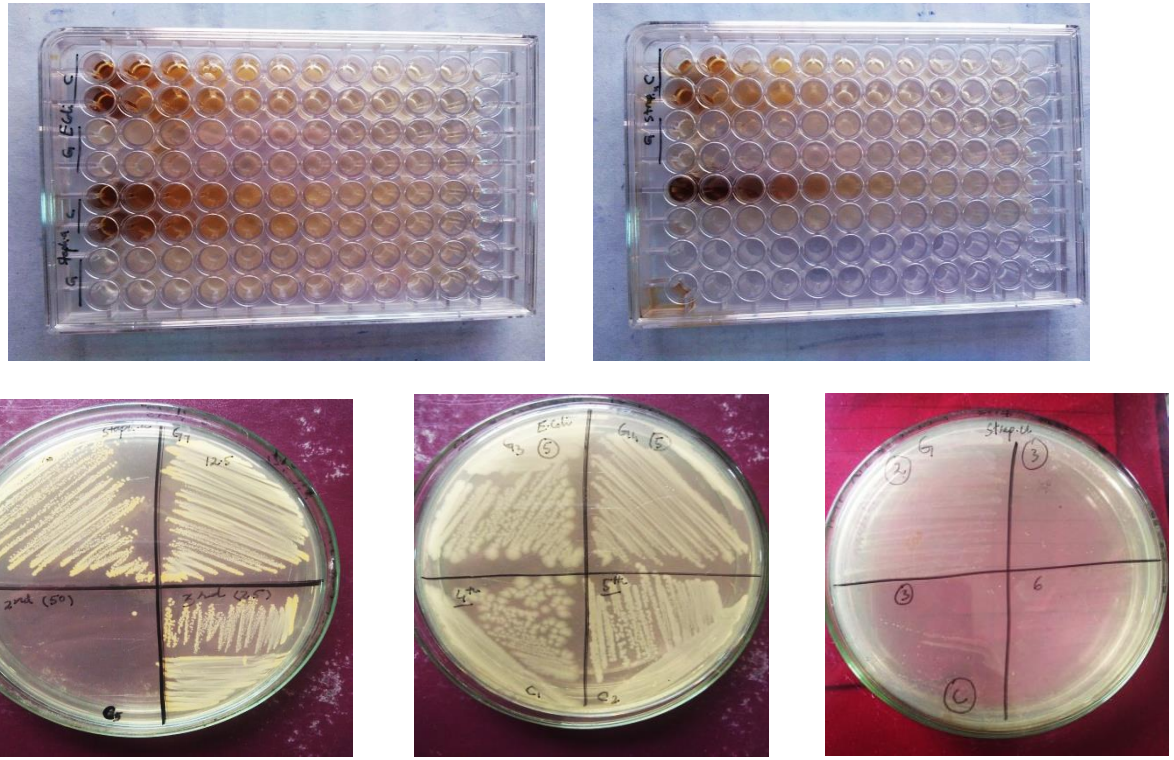
Results and discussion (Cont.)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against mastitogens

A. sativum (Garlic) extract displayed bactericidal effect against *S. aureus* at concentration of 50ug/ml and bacteriostatic effect against *S. uberis* and *E.coli*. Whereas, *C. album* (Bathua) extract showed bacteriostatic activity against all the three mastitogens

Mastitogen	Minimum inhibitory concentration (MIC) µg/ml±SD	
	A. sativum extract	*C. album* extract
Staphylococcus aureus	16.29±8.363	20.04±6.791
E.coli	6.895±3.464	16.29±8.363
Streptococcus uberis	20±6.846	8.75±9.21

Results and discussion (Cont.)



Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against mastitogens (Photo courtesy : Ms. Noor Ul Absar)

Conclusions

Extracts *of Allium sativum* and *Chenopodium album* have antibacterial activity against the mastitogens i.e. *Staphylococcus aureus, E.coli* and *Streptococcus uberis*

Combination of plant extracts show synergistic effect against mastitogens especially against *E. coli*

Acknowledgments

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