Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/4q88fe78

EFFECT OF SEED EXTRACT OF MILK THISTLE SILYBUM MARIANUM L. ON SOME BACTERIAL ISOLATES

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ABSTRACT

Silybum marianum (L). Gaertn is a herb which may be annual or biennial. It is widely distributed in sub-tropical and temperate areas of the world. *Silybum marianum* is commonly known as "Milk Thistle". It belongs to family Asteraceae and contains various flavonolignans; most important of them is silybin. These flavonolignans are medicinally important as they are used as anti-toxins, antispasmodic, anti-allergic, anti-oxidants and anti-fungal agents etc. The plant is also grown commercially in various parts of the world. Various studies have shown that extract of the plant has antibacterial activity against various species of bacteria. Two bacterial species *Staphylococcus aureus* and *Camomonas kerstersii* were tested for the antibacterial activity of the seed extract from the plant through agar well diffusion method. Seed extract of the plant showed high antibacterial activity against gram positive bacterium *Staphylococcus aureus* with a zone of inhibition of 8.0 mm^2 for the aqueous extract with a concentration of 100 mg/ml and 12.0 mm^2 for the methanolic extract with a strength of 100 mg/ml while gram negative bacterium *Camomonas kerstersii* was found to be resistant against the methanolic as well as aqueous extract. An antibiotic Gentamycin used as a control to check the effectiveness of the experiment which showed a zone inhibition of 18.0 mm^2 for the *Staphylococcus aureus* and 17.0 mm^2 zone of inhibition for *Camomonas kerstersii*. Our study has shown that the methanolic extract from the Milk thistle seeds was more effective against *Staphylococcus aureus* and less effective against *Camomonas kerstersii*. *Silybum marianum* may be used as an effective antibacterial agent, was shown by this study.

Keywords: *Flavonolignans*; *Silybin*; Antispasmodic; Antibacterial; Seed Extract

INTRODUCTION

Milk thistle (*Silybum marianum* L.*)* is a herb which may be annual or biennial (Young et al. 1978). It is widely distributed in sub-tropical and temperate areas of the world. Milk thistle belongs to family Asteraceae and contains various flavonolignans. It also has been grown commercially in Europe, Egypt, Argentina and China. The plant is competitive in nature and removes other plant species by shading or competition for nutrients and water (Berner et al. 2002).

It is found along roadside and waste areas forming dense stands and prefers fertile soils (Gabay et al. 1994). The main components of the plant are flavonolignans which are collectively called silymarin (Morazzoni and Bombardelli, 1995). These flavonolignans are medicinally important as they are used as anti-toxins, antispasmodic, anti-allergic, anti-oxidants and anti-fungal agents etc. (Elsayed et al., 2019).

Now a day's milk thistle is utilized as medicinal plant to produce silymarin which is very effective for liver diseases. Silymarin may also be used for prevention from chemotherapy and radio therapy induced toxicity (Abenavoli et al., 2010). *Silybum* also used as fodder for additional nutrient and supplement for animals (Radko and Cybulski, 2007). Seed flour of *Silybum* is also used for young ruminants in Poland (Potkanski et al., 1991). *Silybum* is also used in cosmetic industry due to its high antioxidant activity (Singh & Agarwal, 2009). *Silybum* can also protect skin against UV activity of sunlight (Li et al., 2004). Although silymarin is not easily absorbed in the skin a complex called phytosome is formed by mixing silymarin with phospholipids which can easily pass cell membrane (Semalty et al., 2010).

The plant can easily grow in soils with heavy metals contamination (Del Rio-Celestino et al., 2006) and can absorb these metals from soil to the different plant parts but studies have shown that seed extract of the plant is free from any heavy metal (Brunetti et al., 2009). Milk thistle (*Silybum marianum*) is an annual plant from the Asteraceae family. This plant is native to the Mediterranean and some parts of the United States but is now cultivated in other hot and dry area. The main constituents of the plant are a mixture of flavonolignans with the general name of silymarin, with high antioxidant effects. Silymarin seeds contain about 70–80 % of flavonolignans and 20–30 % of undefined phenolic compounds (Rayni et al., 2023).

Phytochemistry analysis revealed an abundance of active constituents, particularly silymarin, composed of polyphenols and flavonolignans. Silymarin is majorly found in leaves, seeds, and fruits and is comprised of seven flavonolignans. Silymarin derivatives, specifically silybin, were reported for their medicinal properties (Nawaz et al., 2023).

Main constituents of the plant are sugars, proteins, amino acids, alkaloids, saponin tannin and phenolic compounds (Krishnaiah et al., 2007). Accumulation of bio active compounds in plant tissues and synthesis depends on the environmental conditions (Selmar et al., 2013). Due to severe increase of pathogenic resistance in human and other living things it is necessary to find out some natural alternative source from the extract of Milk Thistle plant. Keeping this in view purposed study has following objectives.

1.1 Objectives

Proposed study has following objectives:

1. To prepare the extract from the seeds of *Silybum marianum*
2. To determine the antibacterial activity of the extract against selected bacterial species
3. To study the effect of different extracts on selected bacterial species

REVIEW OF LITERATURE

Enteric bacterial strains cause different diseases and these strains possess antibacterial resistance ability. Different concentrations of the extract from *Silybum marianum* were used to check the antibacterial activity of the extract. Result showed that Silymarin is not useful compound against gram negative enteric bacteria (Mojgan and Roya, 2006).

Physiologically active compounds derived from plants are non-essential nutrients termed as phytochemicals. The aqueous and crude ethanolic extracts from *Silybum marianum* was subjected for antimicrobial activity. Data showed that phenols and tannins were present in the extracts while

saponin and alkaloid were not detected. Silymarin from the extract was found marianum to be very active against gram positive bacteria while no activity was shown against gram negative bacteria (Shah et al., 2011). Organic fertilizers can increase the absorption heavy metals in the plant ten folds than soil. Storage of these heavy metals occurred mostly in the leaves. Research showed that intense absorption and accumulation of heavy metals were seen in the different parts of the plant *Silybum marianum* (Razanov et al., 2020). Patients with side effects due to statins were enrolled for the study. Statins were stopped for one month and *Silybum marianum* extract was given as an adjunctive therapy. Extract reduced the fasting glucose and insulin levels. *Silybum marianum* can be used as supplement to statins for patient unable to tolerate high doses of these drugs (Derosa et al., 2015).

Silybum marianum plant treated with laser which increased the production of silybin A and B and also improved the medicinal properties. *Silybum marianum* extract showed a broad spectrum antimicrobial activity against selected species of bacteria when plant applied with a laser treatment (Aldayel, 2023). Different doses of extracts from *Silybum marianum* were screened for antibacterial activity against two grams positive and two-gram negative species of bacteria. Ethanolic extracts showed more antibacterial activity than aqueous extracts (Puri et al., 2014).

Methanol and chloroform extracts of *S. marianum* collected from ten distinct regions of Pakistan have been found effective against *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, and *Vibrio cholerae* at various concentrations (Ahmad et al., 2015). Ethanolic and methanolic extracts of *S. marianum* found to be very effective against selected bacterial strains while using antibiotics Ampicillin and Amikacin as reference drugs and against different species of genus candida while using reference antifungal agent Fluconazole (Mohammed et al., 2019).

Mixing of extract showed antibacterial activity against bacterial isolates with a concentration ranging from 1500 to 2900 microgram/ml. The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) showed that extract was very active against selected bacterial species (Abed et al., 2015). Among all assayed organic extracts only dimethyl formamide presented highest activities against all tested strains except resistant *E.coli*. Dimethyl formamide and methanol showed better activity than cephradine against ACTT 13581.

The dichloromethane extract showed no antibacterial activity against any strains. Methanol and Isopropyl alcohol showed moderate activities against all tested strains. *S. marianum* would be an interesting topic for further study and possibly for an alternative treatment for resistant and pathogenic bacterial infections (Bajwa et al., 2016).

The results from the high-performance liquid chromatography (HPLC) separation indicated that the phenol, flavonoid, and flavanol contents varied among the four plant samples. Notably, phenol content demonstrated a stronger correlation with antioxidant activity, while flavonoid content was more effective against microbial activity (Geumari et al., 2020). The essential oil yield from Milk thistle seeds was 1.1% (v/w), with eight constituents accounting for 97.3% of the total oil extract. The predominant compounds were oleic acid (45.6%), linoleic acid (29.0%), ethylbenzene (7.0%), and stearic acid (5.7%). The essential oils derived from these seeds significantly inhibited the growth of Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram-negative bacteria, including *Escherichia coli* and *Streptococcus sp.* ($p < 0.05$). Additionally, the oils exhibited fungicidal activity against *Candida* species (Dogan et al., 2022).

The results clearly showed that maximum effect was nearly 77% with 500 μg/mL of ethanolic extract of *S. marianum* after 60 min. Moreover, the scolicidal effects of *S. marianum* extract concentrations were significant in comparison to the controls in all exposure times. It is noteworthy that most of the effect on PSCs was seen within in the first 5 min and showed a linear dose relationship. Beyond that time, there was approximately 30% additional effect over the subsequent 55 min for all concentrations

(Taghipour et al., 2021). Chemical compounds exhibited by *S. marianum* showed various biological activities, as well significant uses in industries. Methyl stearate is a fatty acid methyl ester used as a nonionic surfactant, to enhance the solubility of different chemicals, a stabilizer and an emulsifier. However, the published literature is limited on methyl stearate. Dibutyl phthalate is an important plasticizer also used as a solvent for dyes (Javeed et al., 2022).

Methanolic extract of milk thistle seeds was effective against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica*, *Proteus mirabilis* and *Escherichia coli* but had less inhibitory effect compared to Gentamicin and Ampicillin. In disk diffusion, *Staphylococcus aureus* had the highest inhibitory zone and the highest MIC and MBC. In *Bacillus cereus* and *Salmonella enterica*, the growth inhibition zone diameter was approximately equal to that of *Staphylococcus*, and *Proteus* and *Escherichia coli* showed a smaller diameter zone than *Staphylococcus*. It seems that milk thistle methanolic extract contains antiseptic substances with antibacterial effects (Mahmoudi et al., 2022). Milk thistle seeds were frozen at 0°C for 24 h. Seeds (200g) were powdered and defatted using petroleum ether and Soxhlet apparatus. Then, defatted seed powder was added to hydroalcholic solvent (80%) and soaked for 24 h. Solution was filtrated and the procedure was repeated for two times. Filtrate was mixed well and the solvent was evaporated using rotary evaporator machine. The residue was collected and dried by incubation at 37°C and then was powdered and preserved in dark containers for further use (Jozve-Zargarabad et al., 2020).

Microorganisms against the samples were determined by the agar well diffusion method. The antibacterial activity of plant extracts was evaluated using the diameter of the inhibition zone (mm) around the discs. The results indicated that *U. dioica* extract exhibited activity against *S. aureus*, but did not show any activity against *B. cereus, S. typhimurium,* and *E. coli*. Surprisingly, no antibacterial activity was found in *S. marianum* extract. *C. scolymus* extract only showed antibacterial activity against *B. cereus* (Yılmaz et al., 2022). Twenty-five grams of *Sylibum marianum* seeds powder was placed in each sterile flask with 250 ml of, 70%, and 80% alcohol solution of ethanol, and water, respectively. Then all flasks were incubated in a dark and rotating shaker machine for two hours, the speed of the device was 200 r/ m at room temperature. Filtration was carried out for each sample using filter paper to obtain a pure solution. Then, the supernatants were concentrated by low-pressure rotational evaporation. Evaporation was performed for, ethanol solution, and distilled water separately in the evaporator (Hashem and Kadum, 2022).

MATERIALS AND METHODS

3.1 Sample collection and weighing

Sample of seeds of the plant milk thistle (*Silybum marianum*) was collected in a polythene bag from the territories of the Sialkot, Punjab. Sample was weighed for the fresh weight which was 500grams and subjected to shade dry without exposing it to the direct sunlight. Different number of samples were collected from different locations and listed in the table below;

Table 3.1 Locations, Numbers and selected samples

Sr. No.	Area of sample collection	No. of total samples	No. of selected samples
1	Marakiwal	10	05
2	Sialkot	10	05
3	Uggoki	10	05
4	Bhagal	10	04
5	Waryo	10	03
Total		50	22

Fig. 3.1 Collection of plant Milk Thistle from Marakiwal, Sialkot

3.2 Preparation of extract

For the preparation of extract sample was again measured for the dry weight which found to be 480grams. Then the seeds were crushed using mechanical grinder to form fine powder. Then 50gram of the powder dissolved in methanol and 50gram of the powder was dissolved in distilled water. Then both the solutions were filtered with using filter paper. The solutions were poured in petri plates and allowed to evaporate for 3-4 days. After evaporation petri dishes were scrapped with blade and the extracts were transferred to eppendorf tubes (Shah et al., 2011).

Fig. 3.2 Preparation of extract from seeds of Milk Thistle

3.3 Preparation of concentrations

After that, 5 different concentrations of each extract were prepared by mixing the extract with distilled water having concentrations 1mg/ml, 5mg/ml, 10mg/ml, 50mg/ml and 100mg/ml (Baron et al., 1990).

Table 3.2 Formation of different concentrations from two different solvents

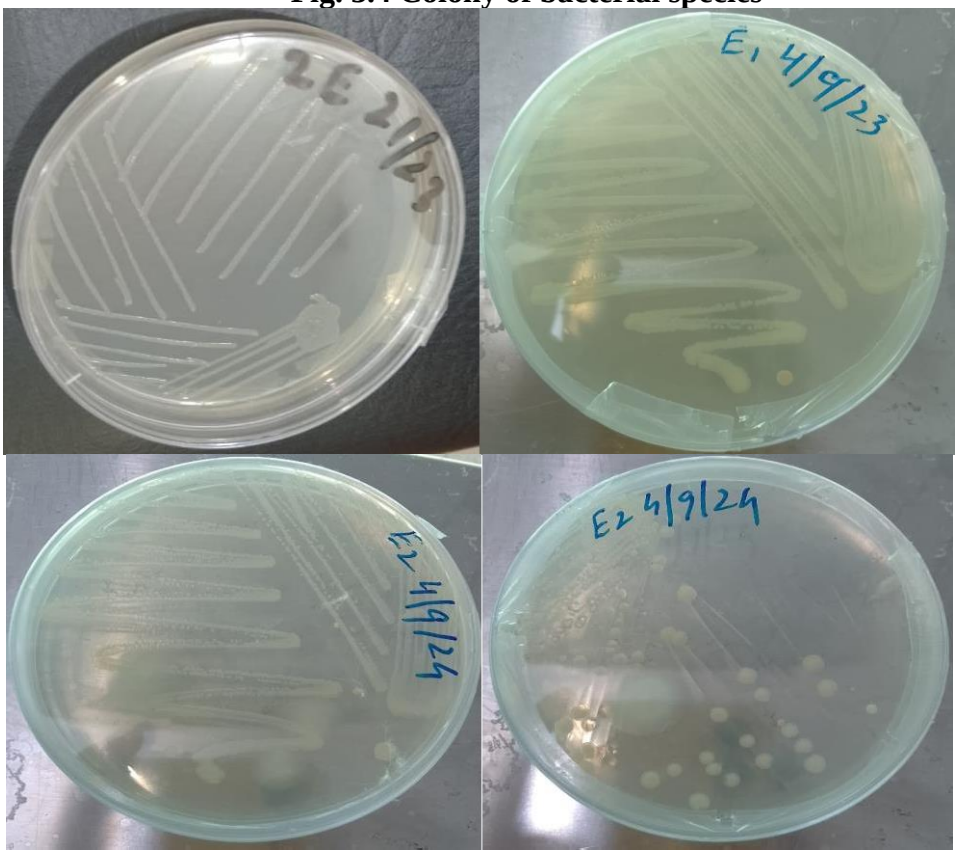
Extract	Methanol	Distilled water	Strength(mg/ml)
7mg	7ml	7ml	1mg/ml
35mg	7ml	7ml	5mg/ml
175mg	7ml	7ml	10mg/ml
350mg	7ml	7ml	50mg/ml
700mg	7ml	7ml	100mg/ml

Fig 3.3 Preparation of concentrations from the extract

3.4 Bacterial species

Two bacterial species *Staphylococcus aureus* and *Camomonas kerstersii* was collected from the Pathology Department of Khawaja Muhammad Safdar Medical College and was cultured according to their standard conditions by using nutrient agar media.

Fig. 3.4 Colony of bacterial species



3.5 Preparation of standard bacterial suspension

Standard bacterial suspensions were formed by using surface viable counting technique. The (10^3-10^4) colony forming units was used by preparing fresh stock solution each time (Shah et al., 2011).

3.6 Test for antibacterial activity

The agar well diffusion method was used to determine the antibacterial activity of the prepared concentrations of the extracts. In which 60ml of the nutrient agar solution was mixed with 0.6ml of bacterial stock solution and 20 ml of the solution was poured in a petri dish. After pouring solution

was allowed to solidify; and five wells was formed by using sterile cock borer no. 7. Agar discs were removed and each well was filled with the prepared extracts one with each concentration by using micro pipette. Then the extracts were allowed to diffuse at room temperature and then petri dishes were incubated at 37°C temperature for 24 hours (Egorov, 1985).

3.7 Evaluation for Minimum Inhibitory Concentrations and Minimum Bactericidal Concentration

The Minimum Inhibitory Concentrations (MIC) was determined by mixing the seed extract with nutrient agar media. The concentrations of the seed extract was in the range of 10^3-10^4microgram per milliliter of the solvent. These concentrations was mixed with melted agar medium and 100 micro-liters of the bacterial suspension also inoculated in the medium and was incubated at 37°C for 24 hours (Abed et al., 2015). The lowest concentrations which completely inhibit the bacterial growth were termed as MIC value. For the determination of the MBC the lowest concentrations which show no visible bacterial growth after sub culturing was considered (Baron et al., 1990).

3.8 Statistical Analysis

All the results and activities from the experiment were proved using evaluative software and Statistical analysis of the study results was conducted using SPSS software.

RESULTS

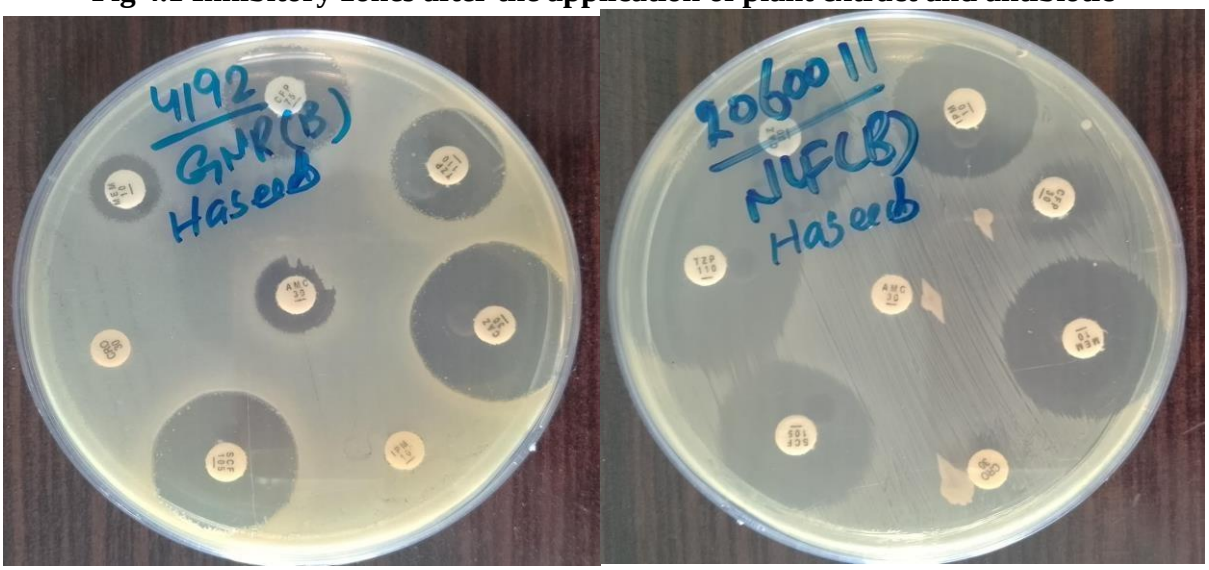
4.1 Evaluation of antibacterial activity

Results from the study told that both methanolic and aqueous extracts showed activity against the gram positive bacteria *Staphylococcus aureus* while gram negative bacteria *Camomonas kerstersii* found resistance towards both extracts which showed no zone of inhibition against any of the extract.

4.2 Evaluation for minimum inhibitory concentrations and minimum bactericidalconcentration

The Minimum Inhibitory Concentrations (MIC) which inhibits the growth of *Staphylococcus aureus* was 10mg/ml and value of Minimum Bactericidal Concentration (MBC) was determined at concentration of 50mg/ml for the aqueous extract, MIC for the methanolic extract was 5mg/ml and the value of MBC was 10mg/ml while *Camomonas kerstersii* remains resistant for both extracts i.e. aqueous and methanolic (Baron et al., 1990).

Fig 4.1 Inhibitory zones after the application of plant extract and antibiotic



4.3 Analysis of aqueous extract zone of inhibition

The aqueous extract concentration against bacteria *Staphylococcus aureus* and *Camomonas kerstersii* had different zones of inhibition with Gentamycin an antibiotic was used for control. The zones of inhibition of bacterium *Staphylococcus aureus* with aqueous concentrations are shown in table 4.1 and 4.2.

Table 4.1 Aqueous extract against bacterial species

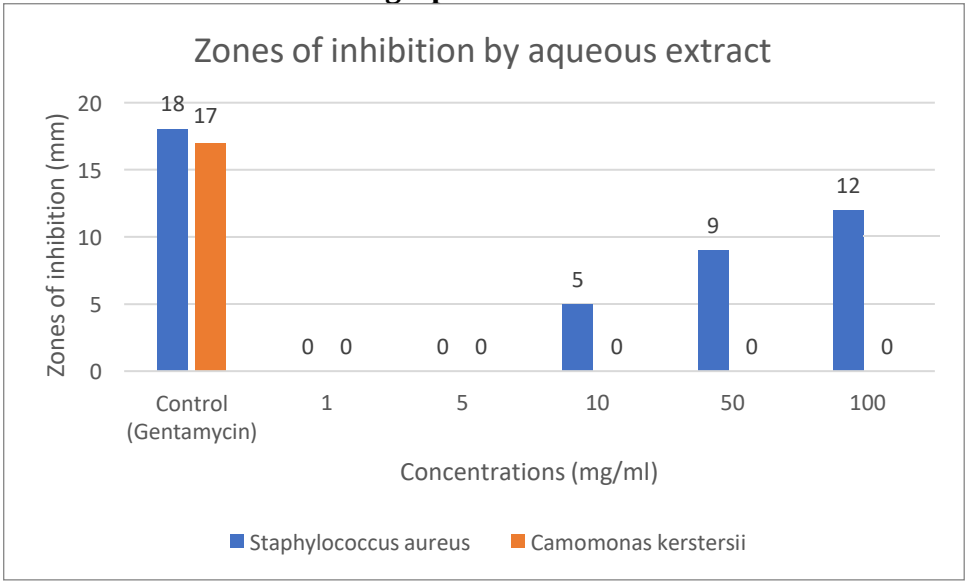
Concentrations	Treatment	Replication	Zones of inhibition($mm^{2)}$	
			S.aureus	*C.kerstersii*
1mg/ml	1	1	0	0
	1	2	0	0
	1	3	0	0
			0	0
5mg/ml	1	1	0	0
	1	2	0	0
	1	3	0	0
			0	0
10mg/ml	1	1	2	0
	1	2	4	0
	1	3	3	0
			3	0
50mg/ml	1	1	4	0
	1	2	5	0
	1	3	6	0
			5	0
100mg/ml	1	1	8	0
	1	2	6	0
	1	3	10	0
			8	0

Most effective concentration which inhibit the growth of bacteria = 100mg/ml
Least effective concentration which inhibit the growth of bacteria = 10mg/ml

Table 4.2 Extract against bacterial species taking Gentamycin as control

Aqueous extract solution	**Mean zones of inhibition(mm^2)**			
	S. aureus	**Gentamycin (Constant conc.)**	***C. kerstersii***	**Gentamycin (Constant conc.)**
1mg/ml	0	18	0	17
5mg/ml	0		0	
10mg/ml	5		0	
50mg/ml	9		0	
100mg/ml	12		0	

Fig. 4.2 Evaluation of zone of inhibition by aqueous extract of plant Silybum marianum by graphical data



Aqueous extract showed greater zone of inhibition (12.0mm^2) against *S. aureus* at higher concentration (100mg/ml), while *C. kerstersii* has not affected by aqueous extract concentration of *Silybum marianum* hence, no zone of inhibitions are formed. Gentamycin affected both species

4.4 Analysis of methanolic extract zone of inhibition

The methanolic extract concentration against bacteria *Staphylococcus aureus* and *Camomonas kerstersii* had different zones of inhibition with Gentamycin an antibiotic was used for control. The zones of inhibition of bacterium *Staphylococcus aureus* with methanolic extract concentrations are shown in table 4.3 and 4.4

Table 4.3 Methanolic extract against bacterial species

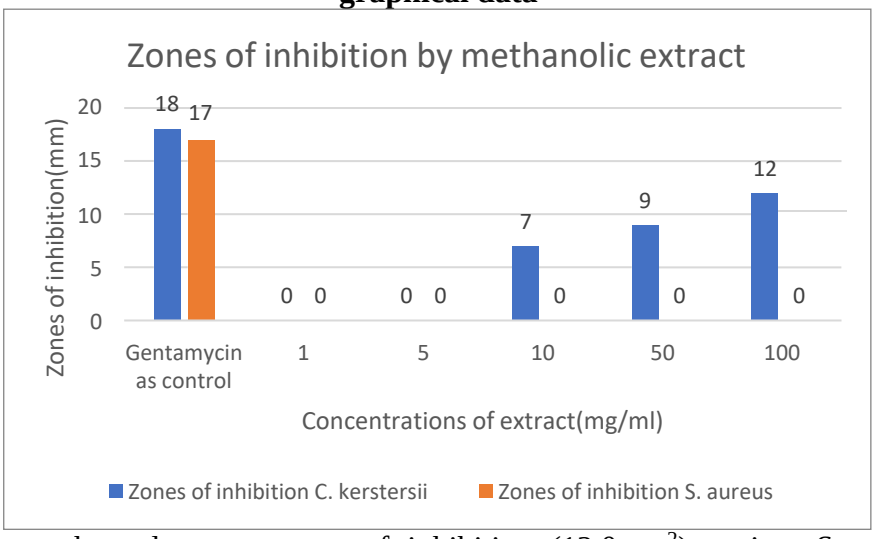
Concentrations	Treatment	Replication	Zones of inhibition(mm^2)	
			S.aureus	*C.kerstersii*
1mg/ml	1	1	0	0
	1	2	0	0
	1	3	0	0
5mg/ml	1	1	3	0
	1	2	5	0
	1	3	2	0
			5	0
10mg/ml	1	1	8	0
	1	2	7	0
	1	3	6	0
			7	0
50mg/ml	1	1	8	0
	1	2	10	0
	1	3	9	0
			9	0
100mg/ml	1	1	14	0
	1	2	11	0
	1	3	11	0
			12	0

Most effective concentration was = 100mg/ml
Least effective concentration was = 5mg/ml

Table 4.4 Methanolic extract against bacterial species taking Gentamycin as control

Methanolic concentrations	Bacterial Zones of inhibition(mm^2) Gentamycin as control with constant concentration			
	S. aureus	**Gentamycin**	***C. kerstersii***	**Gentamycin**
1mg/ml	0	18	0	17
5mg/ml	0		0	
10mg/ml	7		0	
50mg/ml	9		0	
100mg/ml	12		0	

Fig 4.3 Evaluation of zone of inhibition by methanolic extract of plant Silybum marianum by graphical data



Methanolic extract showed greater zone of inhibition (12.0mm^2) against *S. aureus* at higher concentration (100mg/ml), while *C. kerstersii* has not affected by methanolic extract concentration of *Silybum marianum* hence, no zone of inhibitions is formed. Gentamycin affected both species

4.5 Statistical analysis

All the experiments were carried out at different concentrations, each with three replicates and the entire experiment was repeated thrice and data were analyzed by using SPSS software.

4.5.1 Aqueous extract analysis

Table 4.5(a) Mean Analysis for aqueous extract concentration Group Statistics

Extraction type	N	Mean	Std. Deviation	Std. Error Mean
S.aureus	5	3.40	3.209	1.435
C.kerstersii	5	.00	.000	.000

Table 4.5(b) Independent test for aqueous extract concentration

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	11.773	.009	2.369	8	.045	3.400	1.435	.090	6.710
Equal variances not assumed			2.369	4.000	.077	3.400	1.435	-.585	7.385

Null hypothesis: There is insignificant difference between average zones of inhibition for *S. aureus* and *C. kerstersii*.
Alternative hypothesis: There is a notable difference in the average zones of inhibition for *S. aureus* and *C. kerstersii* when using the methanolic extract.
There is significant difference between average zones of inhibition for *S. aureus* and *C. kerstersii* against aqueous extract. According to above table the p value for independent samples test is 0.045, which is smaller than 0.5, so the null hypothesis has been rejected, indicating a significant difference in the average zones of inhibition between *S. aureus* and *C. kerstersii* when exposed to the aqueous extract.

4.5.2 Methanolic extract analysis

Table 4.6(a) Mean Analysis for methanolic extract concentration

Group Statistics

Extraction Type	N	Mean	Std. Deviation	Std. Error Mean
S.aureus	5	5.60	5.413	2.421
C.kerstersii	5	.00	.000	.000

Table 4.6(b) Independent test for methanolic extract concentration

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	23.825	.001	2.313	8	.049	5.600	2.421	.018	11.182
Equal variances not assumed			2.313	4.000	.082	5.600	2.421	-1.121	12.321

Null hypothesis: There is insignificant difference between average zones of inhibition for *S. aureus* and *C. kerstersii*.

Alternative hypothesis: there is significant difference between average zones of inhibition for *S. aureus* and *C. kerstersii* against methanolic extract.

There is significant difference between average zones of inhibition for *S. aureus* and *C. kerstersii* against methanolic extract. According to above table the p value for independent samples test is 0.049, which is smaller than 0.5, so the null hypothesis is rejected and it is concluded that there is significant difference between average zones of inhibition for *S. aureus* and *C. kerstersii* against methanolic extract

DISCUSSION

Milk thistle (*Silybum marianum* L.*)* is a herb which may be annual or biennial. It is widely distributed in sub-tropical and temperate areas of the world. It is found along roadside and waste areas forming dense stands and prefers fertile soils. The main components of the plant are flavonolignans which are collectively called silymarin. These flavonolignans are medicinally important as they are used as anti-toxins, antispasmodic, anti-allergic, anti-oxidants and anti-fungal agents etc. (Elsayed et al., 2019). Now a day's milk thistle is utilized as medicinal plant to produce silymarin which is very effective for liver diseases. Silymarin may also be used for prevention from chemotherapy and radio therapy induced toxicity (Abenavoli et al., 2010). Milk thistle (*Silybum marianum)* seeds were used to form two different extracts. The extracts were then used to check their effectiveness on the selected bacterial species. The aqueous extract of the plant seeds effected the bacterium *Staphylococcus aureus* with the lowest effective concentration of 10mg/ml with a zone of inhibition $3mm^2$ and moderate effective concentration 50mg/ml with a zone of inhibition $5mm^2$ and the most effective concentration 100mg/ml with a zone of inhibition of $8mm^2$ while bacteria *Camomonas kerstersii* found to be resistant against all the concentrations of the aqueous extract showing no zone of inhibition. The methanolic extract found to be effective against *Staphylococcus aureus* showing the lowest effective concentration of 15mg/ml with a zone of inhibition of $5mm^2$ and the moderate effective concentration was 50mg/ml which showed the zone of inhibition of $9mm^2$ and the most effective concentration was 100mg/ml with the zone of inhibition of 12 mm^2 while *Camomonas kerstersii* found to be resistant towards the methanolic extract for all the concentrations which correlate with the previous results of Nanpazi et al., 2020. Results of the study revealed that methanolic extract was more effective against the *Staphylococcus aureus* than the aqueous extract which relate with the results of Mahmoudi et al., 2022.

CONCLUSION

The bacterium *Staphylococcus aureus* had larger zone of inhibition than *Camomonas kerstersii* for both the extracts. Hence, larger inhibition zone mean that plant *Silybum marianum* (Milk Thistle) has severely affected the pathogenic bacteria, so the plant extract has proved to be a very effective bio-control agent against the *Staphylococcus aureus*.

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