

Integrated Chemical and Microbiological Study of *Sonchus Oleraceus* as a Natural Antibacterial Agent

Saly Naser Abbas ^{a*}, Fatimah Abdul Razzak Mageed ^b, Ali Hussein Kadhim ^c, Thoalffakar A. Alhamed ^d

^{a*,b,c,d} Department of biology, College of Education for pure Science, University of Kerbala, Kerbala, Iraq

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ABSTRACT

Antimicrobial resistance is becoming a growing serious health problem worldwide and the utilization of medicinal plants has been recognized as an important source of new antimicrobial agents that has become attractive. *Sonchus oleraceus* is a traditional medicinal plant utilized in folk medicine for the treatment of infections, inflammation and various microbial diseases. Hence, the present study was designed to study the phytochemical composition and activity antibacterial of *Sonchus oleraceus* extracts against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus*. Different group of bioactive compounds were isolated from aerial parts of the plant by using chloroform, methanol and distilled water. Phytochemical screening such as phenolic compound, flavonoid, alkaloid and terpenoids was performed on the extracts to determine the main secondary metabolites, which are biologically and antimicrobially important. The extracts were tested for their antibacterial activity by disk diffusion method at concentrations 50 and 100 mg/mL and for the ciprofloxacin was used as a positive control. Concentrations (50 and 100 mg/mL) of the extracts were used to assess their antibacterial activity against the tested pathogens using the disk diffusion method, with ciprofloxacin used as the positive control. The extracts were unable to inhibit the growth of *Escherichia coli* but the chloroform extract exhibited antibacterial activity against *Staphylococcus aureus* at 100 mg/mL against *Staphylococcus aureus* at 100 mg/mL. These results are significant as they indicate a selective activity of the plant extracts, specifically on Gram-positive bacteria as potential presence of specific bioactive compounds with targeted antimicrobial activity. The differences in antibacterial activity of the extracts may be due to the differences in the chemical composition, polarity and solubility of the phytochemicals extracted by each extract solvent respectively. In conclusion, the study underscores the possibility of *Sonchus oleraceus* as a rich source of Bioactive metabolites exhibiting considerable potential pharmacological applications. The activity against bacteria was limited, in spite of the fact that it showed selective activity along with a variety of phytochemical components that offers a solid foundation for further studies to isolate, purify and characterize the active compounds that achieve these biological effects. Further research using advanced analytical methods (GC-MS), higher concentration of extracts, and other extraction methods is suggested to gain knowledge about the therapeutic applications of this plant as a natural antibacterial.

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1. INTRODUCTION

1.1 Bacterial infection

Bacteria are ubiquitous, they play an important role in maintaining the environment in which we live; few bacteria species are pathogenic and cause infections and diseases. These bacterial infections have a large impact on public health (Doron & Gorbach, 2008). Generally, bacterial infections are easier to treat than

*Corresponding Author Institutional Email:
sally.n@uokerbala.edu.iq (Saly Naser Abbas)

viral infections; the spectrum of antimicrobial drugs with activity against bacteria is much broader than that of infectious diseases caused by viruses or parasites, but the problem of bacterial resistance to antimicrobial agents is rapidly increasing and can have devastating impacts (Doron & Gorbach, 2008).

1.1.1. Bacterial epidemiology

The site of the bacteria and host interaction and infection is typically the external environment. Humans can contract bacteria via air, water, food or living vectors) (Doron & Gorbach, 2008). The macro- or microenvironments can also be considered to be participating in the spread of bacteria. There are special organisms that can be found in places like hospitals and prisons. There are bacteria which are found in a specific geographic region and are not found, or are very rare in other regions (Doron & Gorbach, 2008).

1.1.2 The Structure and Classification of Bacteria

Bacteria are prokaryotic organisms that carry their genetic information in a double-stranded circular molecule of DNA; some species also contain small Extra DNA in the form of circular plasmids. The cell cytoplasm is filled with ribosomes and the cell has a cell membrane, as well as in all species except Mycoplasma a complex cell wall. Some bacteria are provided with pili, flagella or capsules outside of their cell wall (figure 1) (Bannister et al., 1996)

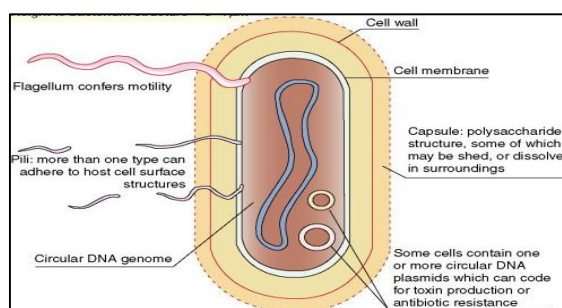


Figure 1. Structure of a bacteria (Bannister et al., 1996) Clinically the main difference between Gram-positive and Gram-negative bacteria is the presence of endotoxin in the latter that can lead to tissue destruction, shock and death, The same is true for the two kinds of bacteria; they are also sensitive to different antibiotics (Doron & Gorbach, 2008). Aerobic bacteria, grow in the presence of oxygen, Obligate aerobes grow only in the presence of oxygen and Facultative organisms grow with or without oxygen, Anaerobic bacteria are able to grow in the absence of oxygen and obligate anaerobes require its absence) (Doron & Gorbach, 2008). "Some bacteria cannot be readily classified as either Gram-positive or Gram-negative because of their unique cell wall structures - These are positive or gram-negative and

they are the mycobacteria of which mycobacterium tuberculosis is the most famous, and the Rickettsia. (Binda et al., 2020)

1.1.3 Bacterial resistance a global concern

Antimicrobial resistance (AMR) does not mean our body is resistant to antibiotics, It indicates that the organism associated with the infection is resistant to the antibiotic used, Antimicrobial medicines are the backbone of modern medicine, As drug-resistant pathogens emerge and spread, Antimicrobial Resistance threatens our capability to treat common infections, to perform lifesaving procedures (cancer chemotherapy, C-section, hip replacement, organ transplant, and surgery), and has significant health system and national economic costs due to the need for more expensive and intensive care, the effect on patients' productivity, for example, or due to longer hospital stays, The misuse and overuse of antimicrobials in humans is the main drivers in the development of drug- resistant pathogens (Collaborators, 2022)

1.2 Herbal medicine: the magic way overcoming microbial resistance

Although antibiotics are used sparingly, new antibiotics would not have a significant effect on the damaging strains of antibiotic resistant bacteria and are likely to increase the potential for antibiotic resistance by bacteria even further (Eldin et al., 2023). Many plants have been found to have strong antimicrobial properties which can be used in a synergistic or synergistic alternative to antibiotics, so the use of herbal medicine has surged in the past few years to combat antibiotic resistant microbes, The best results will be obtained if both the pharmacokinetics and the pharmacodynamics of a natural product are similar to the antibiotic characteristics (Eldin et al., 2023).

1.2.1 Antimicrobial Activity Mechanisms of Medicinal Plant

There is tremendous potential in the discovery of new bioactive compounds from medicinal plants that are able to combat resistant microorganisms (Verpoorte, 2024) Secondary plant metabolism compounds of therapeutic importance are referred as bioactive compounds (phytochemicals) taken from plant and are mostly intermediate or end products of secondary plant metabolism. (Stefanovic, 2012). heir antimicrobial activity spectrum is wide, depending on the structure, number, position of the substituent groups, presence or absence of glycosidic connections, OH group alkylation, topography and climate of origin. Besides, alteration of the quality and quantity of the ingredients is often accompanied by changes in the antimicrobial activity of bioactive plant and sometimes it is mixtures of compounds; synergy can be at work causing the

antimicrobial activity to be amplified (Merkl et al., 2010) (Arima et al., 2002) (Assob et al., 2011). In general, the primary target site of these compounds on the microbial cell is the cytoplasmic membrane where their activity is manifested in various ways (Savoia, 2012) (Radulović et al., 2013) (Saleem et al., 2010) such as affecting the structure and integrity, permeability, or function of the membrane. Moreover, it is suggested that plant extracts might have restraints of Efflux Pump (EP) as a constituent of their composition (Savoia, 2012). Other compounds would change or even inhibit the protein-protein interaction, thus becoming modulators of immune response, mitosis and apoptosis (Vadhana et al., 2015).

Furthermore, they can interfere with intermediary metabolism (Anandhi et al., 2014) to cause cytoplasmic constituents (Mogosanu et al., 2015) to coagulate, inhibit biofilm formation, which is a protective mechanism for pathogens during the infection period (Quave et al., 2012; Talekar et al., 2014) and they can be divided into groups according to their chemistry, chemical composition, biosynthetic pathway or solubility, with a large number of compounds (Kabera et al., 2014; Alamgir, 2017)

1.3 Herbal Medicine: Current Status and the Future

Herbal medicine occupies a prominent place within primary healthcare systems in many societies, particularly in developing countries. This is due to its continued role in providing affordable health services that are compatible with the cultural heritage of the population, making it a popular choice for a wide range of individuals (World Health Organization, 2023). This is mainly due to the common notion that herbal medicines lack any side effects other than being readily available and inexpensive. The World Health Organization (WHO) reported that the use of herbal products on the globe is more than twice the conventional drugs. Most of the few effective drugs of the 100 years ago were derived from plants, such as acetylsalicylic acid (willow bark), digoxin (by foxglove: medicinal plant), quinine (Cinchona bark) / Morphine (derived from the opium poppy, *Papaver somniferous*). According to a survey conducted in 2023, it has been reported that the number of patients using alternate and herbal therapy is increasing rapidly.

At the same time severe adverse reactions have been reported on some herbal drugs, indicating that all herbal medication may not be safe. Most herbal medicines. The traditional use of available over a thousand years may give us guidelines on the selection, preparation and application of herbal formulation. If the herbal product is to be considered as a viable alternative to modern medicine, the same rigorous approach, which is used to take scientific and clinical methods to validate the safety

and effectiveness of a therapeutically viable product must be employed (Pal & Shukla, 2003).

1.4 Medicinal values of herbal plants

Isolation of extracts from medicinal plants has been reported to have different biological activities as antimicrobial, anti-inflammatory and antioxidant (Shankar et al., 2010). Antimicrobial substances got from medicinal plants which have various mechanisms of action and may have clinical significance against microbial strains that are resistant to antibiotics; some of these compounds exhibit intrinsic antibiotic activity and some exhibit antibiotic resistance-modifying activity, while remaining less toxic than synthetic drugs, have higher therapeutic potential and lower possibility of resistance developing (Ruddaraju et al., 2020; Almabruk et al., 2018).

A variety of chemical compounds has been found *in vitro* to have a synergistic inhibiting effect on bacteria growth, which can occur when two or more active components of the medicinal plants are involved in the same bacteria growth inhibiting mechanism, or when one compound in the medicinal plant has an inhibitory effect on the resistance mechanism of the bacteria, or when two or more active components of the medicinal plants are responsible for the same mechanism in inhibiting bacteria growth, or when two or more active components of the medicinal plants are responsible for enhancing the bacteria inhibiting activity of the active component, or when two or more active components of the medicinal plants are responsible for the enhancing of the bioavailability or solubility of the bacteria inhibiting active component exhibit antimicrobial activity in medicinal plants and it is extremely difficult to include all the medicinal plants and the antimicrobial compounds of high antimicrobial potent in this report. However, *Sonchus oleraceus* is one of these compounds of high interest. The same vigorous approach used to test scientific and clinical methods to validate the safety and efficacy of a therapeutically viable product needs to be applied to validate a herbal product, an alternative to modern medicine (Kaundal et al., 2021; Hassan et al., 2014)

1.5 *Sonchus Oleraceus*

In 1753, *Sonchus oleraceus* was named by Carolus Linnaeus, *Sonchus* is Greek for sph enche (a pithy stem), and *setosus oleraceus* a kitchen plant. It is regarded as one of the medicinal troublesome weeds in greater than 55 nations (Resende et al., 2015).



Figure 2. Flower of *Sonchus Oleraceus* (Resende et al., 2015)

Sonchus Oleraceus originally from Europe, North Africa and West Asia, was introduced to North and South America, India, China, Southern Australia; initially a weed and now found in cultivated crops or around roadsides, a plant that had important medicinal uses, recognized later, and is an annual, erect, leafy and glabrous, with white milky juice, taproot up to 1 m high. (Ahmad et al., 2015; Resende et al., 2015; Puri et al., 2018).

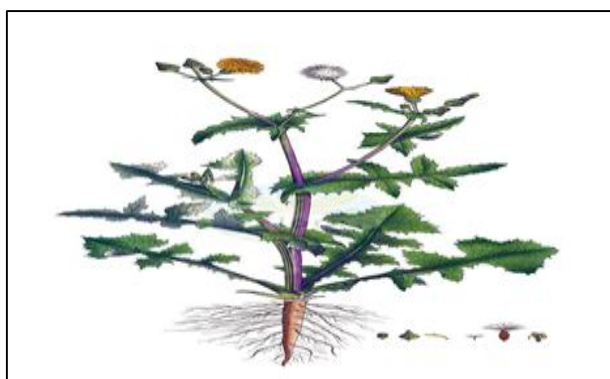


Figure 3. *Sonchus Oleraceus* (Puri et al., 2018)

It is traditionally used as a folk medicine for gastrointestinal tract, as a sedative and as a vermifuge, also in healing of wounds, in treating liver disease and ulcers, a vermicide. A complex mixture of leaves, stems, flowers, roots and seeds of this plant is used in the treatment of vitiligo, as a cathartic and as a treatment of cancer, helps in the treatment of phthisis, treats gastric spasm, helps in the treatment of infections, inflammation, headache, general pain and rheumatism. Leaves and stems are rich in some Pharmacologically active compounds that play an important role in the preparation of herbal products, which are made from mixtures of leaves, stems, flowers, roots, and seeds of this plant are used (Ou et al., 2013; Ou et al., 2015; Hamadnalla, 2020). The secondary metabolites in the whole plant extracts were identified as the major components include terpenes, steroids, flavones and coumarins, along with flavonoids (luteolin, apigenin, kaempferol, and quercetin) which helps protect the plant from the effects of UV radiation and fungal diseases. Carotenoids composition and chemical analysis of coumarin of *Sonchus Oleraceus* was isolated and studied. It is a plant with a high antioxidant content and antioxidant activity, recently identified in its extracts the caftaric acid (Ali, 2020)

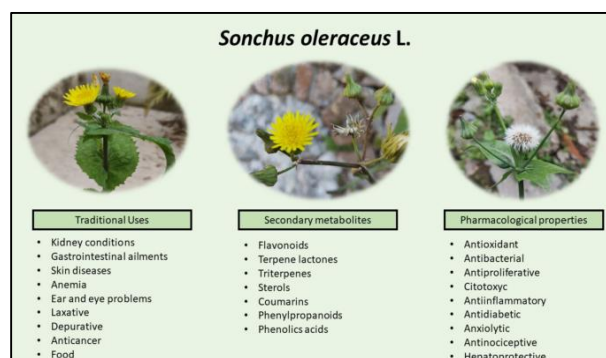


Figure 4. Graphical Abstract of *Sonchus oleraceus* (Ali, 2020)

Table 1. Phytochemical constituents present in different extracts :

Phytochemical Constituents	Aqueous Extract	Methanol Extract	Chloroform Extract
Alkaloids	+	+	+
Carbohydrates	+	+	-
Flavonoids	+	+	+
Tannins	+	+	-
Terpenoids	+	-	+
Phenols	-	-	+
Saponin	+	-	-
Xanthoprotein	+	-	+

Bioactive Phytochemicals Identified in Different Extracts

2. Material and methods :

Sonchus oleraceus was extracted by using three solvents of different polarities and antibacterial activity of these extract were studied by using antimicrobial sensitivity test against one gram -ve bacteria *Escherichia Coli* (*E. coli*) and one gram +ve bacteria *Staphylococcus aureus* (*Staph.aureus*) by using two concentrations of each extract (50 and 100mg/ml) and comparing with positive control (ciprofloxacin 10mcg)

2.1 Materials

2.1.1 Plant collection and authentication

The aerial parts of *sonchus oleraceus* was collected in may- 2023 from Almusyib –Babil- Iraq, the species were identified and authenticated by a professional taxonomic .

2.1.1.1 Extract preparation

Thirty grams of grinded aerial parts of *Sonchus Oleraceus* subjected to soxhlet extraction method using 150 ml of solvents increased in polarity (chloroform and methanol) respectively .

The duration of extraction for each used solvent of 3 hrs. After this, the plant material was transferred to the distilled water (150 ml), which was used for extraction at 60 c for 2 hr. The chloroform and methanolic extract were placed. We used a rotary evaporator to remove the solvents and obtain the dry extract, while the water extract was dried by freeze-drying.

After that, extracts grinded finely and dissolved by using Dimethyl-Sulfoxide (DMSO).

2.1.1.2 Strain of bacteria

In this study the bacteria utilized were:

-gram -ve bacteria (E. coli) and gram +ve bacteria (Staph.aureus) that were collected from dietary product (cheese.)

2.1.2 Chemicals

Highest purity chemical where chosen; tables of these chemicals are demonstrated as the following:

Table 2. Chemicals and their origin.

Chemicals	Manufacturer	Origin
Dimethyl-sulfoxide (DMSO)	ALPHA CHEMIKA	INDIA
Ethanol 99 %	AL JOOD	IRAQ
Chloroform	THOMAS BAKER	INDIA
Muller hinton agar	SETOD.ROBRUZZI	ITALY
Blood agar	BIOMARK LABORATORIES	INDIA

Substances of Chemicals and their origin .

2.1.3 Apparatus and Equipment

Instruments and laboratory appliances used in this study are summarized in the following tables

Table 4. Instruments and their origin.

Instrument	Manufacturer	Origin
Rotatory evaporator	STUART	EUROPE
Sensitive balance	RAYLABEL	CHINA
Fume hood	DAIHAN LABTECH	KOREA
Incubator	BIOBASE	CHINA

The origin of instrument

Table 4. laboratory appliances.

laboratory appliances	
Gloves	Petri dish
Face mask	Soxhlet apparatus
Graduated cylinder	Bunsen burner
Mortar & pestle	Glass beaker
Pipette	Cotton
Volumetric flask	Spatula
Inoculation loop	Forceps

Laboratory equipment

2.2 Methods (Manandhar et al., 2019) ,(Amenu, 2014)

2.2.1 Method of disk diffusion

This method goes that sterile filter paper disc method were placed in tubes with dilutions of extracts (50 and 100mg/ml of chloroform, methanol and water) after 5-10 minutes extracts stucked to disks which left to dry completely then they were ready for applications.

2.2.2 Bacteria culture

We used surface culture of bacteria by swabbing Mueller-Hinton agar plate. under sterilized conditions.

2.2.3 Design of antimicrobial sensitivity test (Mahato and Sharma, 2018)

The disks containing different dilutions of extract were put on the surface of the culture with an appropriate distance from edge of the plate and from each other. Were incubated for 24 hrs at 37 c and results of antibacterial effect were calculated by measuring the diameter of the zone of inhibition.

2.2.4 GC-MS Analysis (Gas Chromatography-Mass Spectrometry)

The chemical composition of the chloroform and methanolic extracts of *S. oleraceus* was determined by Gas Chromatography-Mass Spectrometry (GC-MS) which identified several phytochemical constituents including fatty acids, phenolic derivatives, terpenoids and long-chain hydrocarbons, the major ones being hexadecenoic acid (palmitic acid), phytol and sterol derivatives, which have been reported with antimicrobial and antioxidant activity, The antibacterial activity reported here is attributed to the presence of these compounds, with many fatty acids and terpenoids reported to have an effect on bacterial cell membranes

and microbial growth. The absence of good activity against *Escherichia coli* is, however, due to the complexity of its outer membrane structure which makes it less permeable to the hydrophobic compounds (Chougui et al., 2024).

3. Results and Discussion

3.1 Extracts characteristics:

Table 5: extracts properties

Solvents of extraction	Characteristics	Color	Weight
Chloroform	coarse powder	Green	0.8gm
Methanol	Paste-like	Dark brown	3gm
Distilled water	irregular shape particles	Brown	0.8gm

Derives properties



Figure 6. characteristics of different Sonchus Oleraceus extract

Table 6. Antimicrobial sensitivity test of Sonchus oleraceus against *E. coli*

Extracts	Concentration mg/ml	Zone of inhibition
Chloroform	50	Na
	100	Na
Methanol	50	Na
	100	Na
Distilled water	50	Na
	100	Na

Na: not active (there is no visible inhibition area)

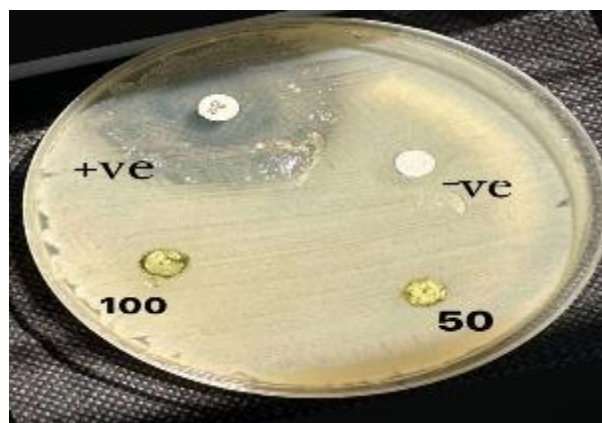


Figure 7. anti- *E. coli* of Sonchus Oleraceus. Chloroform extract

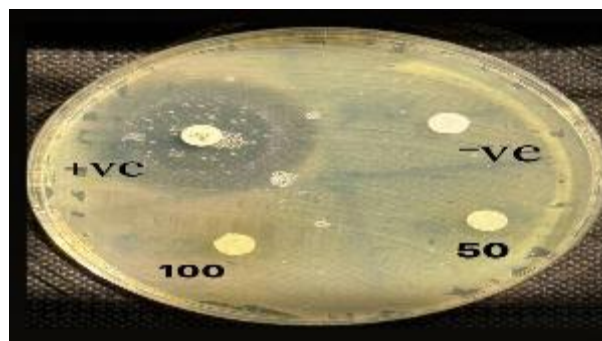


Figure 8. anti- *E. coli* of Sonchus Oleraceus Methanol extract.



Figure 9. Anti- *E. coli* of Sonchus Oleraceus. Water extract

Table 7. Antimicrobial sensitivity test of sonchus oleraceus against *staph.aureus*.

Extracts	Concentration mg/ml	Zone of inhibition in diameters
Chloroform	50	Na
	100	+ve (1mm)
Methanol	50	Na
	100	Na
Distilled water	50	Na
	100	Na

Na: not active (there is no visible inhibition area).
+ve there is visible inhibition

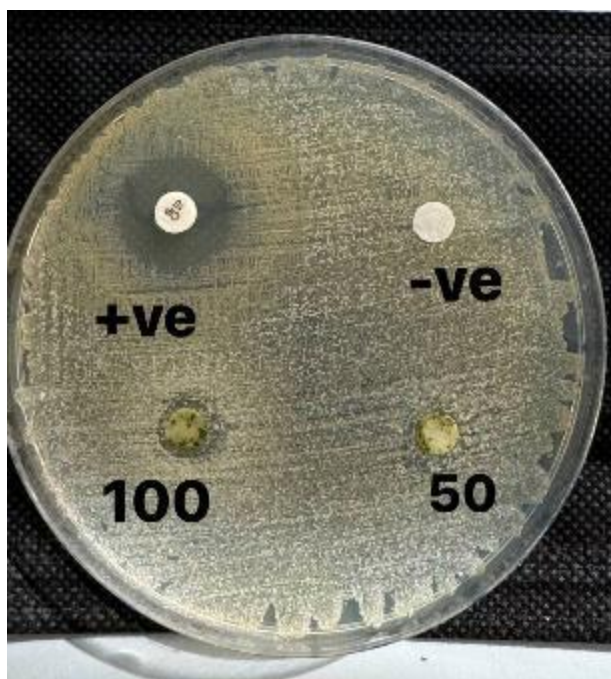


Figure 10. Anti-staph aureus of Sonchus oleraceus, Chloroform extract

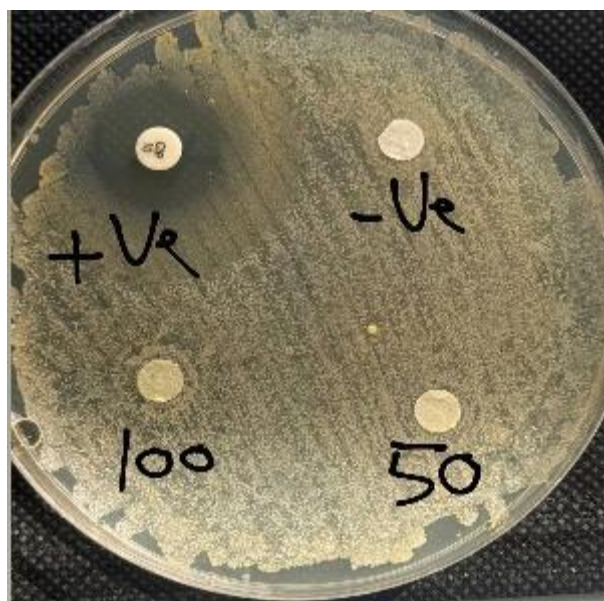


Figure 11. anti-staph aureus of Sonchus oleraceus. Methanol extract

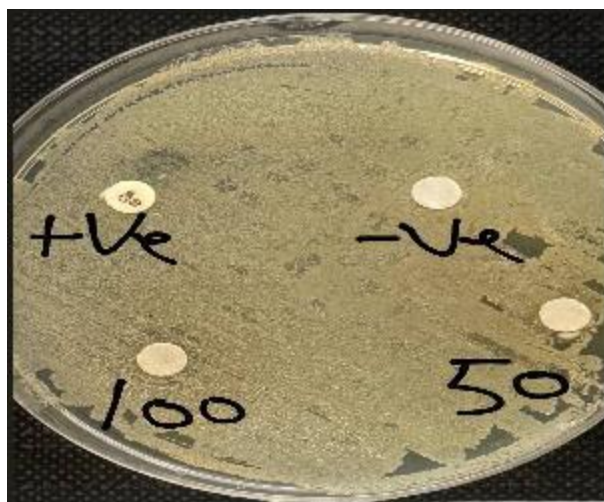


Figure 12. anti-staph aureus of Sonchus oleraceus. Water extract

Table 8. Major Compounds Identified in *Sonchus oleraceus* Extract by GC–MS Analysis

No .	Compound Name	Retention Time (min)	Molecular Formula	Biological Activity
1	Hexadecanoic acid (Palmitic acid)	18.52	C16H32O2	Antibacterial, antioxidant
2	Phytol	21.34	C20H40O	Antimicrobial, anti-inflammatory
3	Octadecanoic acid (Stearic acid)	24.11	C18H36O2	Antioxidant activity
4	Linoleic acid methyl ester	25.	C19H34O2	Antibacterial activity
5	β -Sitosterol	29.72	C29H50O	Anti-inflammatory, antimicrobial
6	Squalene	31.15	C30H50	Antioxidant and protective effects

GC–MS Identification of Major Compounds in *Sonchus oleraceus* Extract

3.2 -Discussion

Medicinal plants have been identified as valuable natural sources of bioactive compounds with therapeutic and antimicrobial properties. The World Health Organization (WHO) considers medicinal plants as important resources for the development of medicines in traditional and modern medicine, The antibacterial effect of the chloroform, methanol and aqueous extracts of *Sonchus oleraceus* was determined against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* by disc diffusion method at 50 and 100 mg/mL, No inhibitory effect was observed against *Escherichia coli* for all the extracts tested, but slight activity was observed for *Staphylococcus aureus*

for chloroform extract with small zone of inhibition as compared to ciprofloxacin at 100 mg/mL.

The observed antibacterial activity was limited but this result confirms that *Sonchus oleraceus* contains biologically active constituents with selective activity against Gram-positive bacteria. The results are somewhat in line with other reports which suggested that the antibacterial activity increases with the concentration of extract, especially for *Escherichia coli* in the aqueous extract. Differences among the studies may be attributed to the variations in the extraction concentration, geographical location, environmental conditions, and climatic conditions involved in the biosynthesis and accumulation of the secondary metabolites in medicinal plants. Temperature, precipitation, altitude, light intensity and other environmental factors are known to affect the production of antimicrobial phytochemicals such as tannins, phenols, flavonoids, terpenoids and alkaloids.

The difference in susceptibility of Gram-positive and Gram-negative bacteria, seen during this study, is due to their different cell wall structure, Gram-negative bacteria are more resistant to plant extracts than Gram-positive bacteria and have an extra barrier, the outer lipopolysaccharide membrane and periplasmic space which hinders penetration of antimicrobial compounds. In addition, past research has shown that the well diffusion method could yield a greater antibacterial activity than the disc diffusion method in the case of better diffusion of the active compounds into the culture media as in Kaundal et al., IJPSR, (2021), but we found there is little visible effect (1mm inhibition zone diameter compared to positive control (ciprofloxacin 10mcg) (5mm) figure (7-10). These findings suggest that further studies using higher extract concentrations may be warranted used. These results are in agreement with [Hatim MY Hamadnalla, et al (2020)] who studied the antibacterial effect of *sonchus oleraceus* against *staph.aureus* using different extract concentrations (100,50 ml) using disk diffusion method to conduct his result. These results agree with the research results.

However, in contrast to our results, Hadi Koohsari, MD states that the anti-bacterial effect of herbal extracts was greater using better method than with the Disk diffusion method. Some similar studies such as the well method performed more inhibitory while the disk diffusion method performed less inhibitory. This may be related to the effect obtained on blank paper disks was less, and the amount of drug molecules released to

the surface of the disk culture of bacteria was also less than the amount of drug molecules released from the well method to the culture (Walter et al., 2011),(Indh et al., 2006),(Dadgar et al., 2006).

The results of the GC–MS findings also corroborated the antimicrobial activity of *Staphylococcus oleraceus*, as several bioactive phytochemicals such as fatty acids, phytol, and squalene with antimicrobial and antioxidant activities were identified.

These compounds could exhibit synergistic effects that cause disturbance of bacterial membranes and inhibit microbial growth, The overall antibacterial activity was found to be low however the presence of the pharmacologically important phytochemicals indicates that *Staphylococcus oleraceus* could be a good source for further investigation of antimicrobial agents at higher extract concentrations and other testing methods.

3.3 Conclusions

The use of medicinal plants as antimicrobial agents is a new possibility to fight the perilous issue of antimicrobial resistance that's surfacing. Hence, there is an urgent need to search out novel bioactive compounds from those medicinal plants which have not been sufficiently explored and exploited. Their rich variety has demonstrated therapeutic potentials included in the field of antimicrobials, and as antimicrobial resistance modifiers. New bioactive compounds have to be used. Yet, the potential use is challenging. It has to be noted here that a lot of in vitro and in vivo trials need to be done to ensure selection of active and nontoxic antimicrobial plant compounds. Medicinal plant extracts contain compounds which have possible synergistic or antagonistic interactions within and between extracts. Are also a great challenge to be exploited. The overall results of the integrated chemical and microbiological analysis suggest that *Sonchus oleraceus* is a good source of phytochemicals that can be used in the pharmaceutical and antibacterial sectors. The future therapeutic development is suggested to include compound isolation, molecular docking and evaluation of cytotoxicity.

3.4 Recommendations

- Evaluate higher extract concentrations to determine whether enhanced antibacterial activity can be achieved Studied extract in different types of bacteria
- Using another part of plant like the root
- Using well diffusion method
- Identified plants to know the active ingredients

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