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Detection And Extraction of Antibacterial Compounds from The Leaves of Sonchus Asper Plant

[1]Rizwana Kausar (Corresponding Author), [2]Lubna Razaq, [3]Faiz Ur Rahman, [4]Muhammad Zafar

[1]Ph.D student; School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.
[2]Departement of Microbiology, Karachi University, Karachi Pakistan.
[3]Department of Microbiology, Quaid-i-Azam University Islamabad, Pakistan
[4]Assistant Professor, Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan
[5]Alyaa Abdulhussein Alsaedi
Ph.D student; School of Industrial Technology, University Sains Malaysia, Penang, Malaysia.

Abstract

Antibiotic resistance among different pathogenic bacteria poses a significant threat to human health. The search for novel class of antimicrobials from different resources such as medicinal plants is a continuous effort of the modern-day scientists. The present work was planned to extract and evaluate the antibacterial activity of *Sonchus asper* crude extracts against multi drug resistant bacterial pathogens. Methanolic, ethanolic and aqueous extracts dissolved in DMSO have been tested against multi drug resistant human's bacterial pathogens, including Gram-positive bacteria *Staphylococcus aureus* ATCC 43300 and Bacillus subtilis subsp. B. spizizinii ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aureginosa* ATCC 27853. The well diffusion method was used by measuring the zone of inhibition of bacterial growth from the plant extracts while DMSO as negative control and streptomycinas a positive control. All three extracts showed good antibacterial activity. However, methanolic extract of *S. asper* showed maximum antibacterial activity against *S. aureus*, while zones of inhibition for B. *subtilis subsp.* B. spizizinii, *E. coli,* and *P. aureginosa* were also significant. Furthermore, FTIR spectroscopic analysis for methanolic extract of *S. asper* showed the presence of various organic bioactive compounds. It is concluded that *S. asper* has crucial medicinal significance, and advanced molecular-level analysis of its components may provide a basis for the production of clinically important natural antimicrobials against various pathogenic and resistant bacterial strains.

Keywords: antibacterial; antibiotic resistance; natural product; *S. asper*;

Introduction

The emergence of bacterial resistance is an issue of great concern as it is seriously threatening human health (Dadgostar, 2019). The evolution of these resistant microorganisms has dramatically compromised the use of newer generations of antibiotics (Kültür, 2007). Pathogenic microorganisms are continually evolving resistance to naturally as well as synthetically manufactured compounds. The most significant challenge to treat these bacterial infections is multidrug resistivity and less susceptibility of bacteria strains towards antibiotics (Walter, 2011). Moreover, the host immune system is also adversely affected by over usage of antibiotics (Willing et al., 2011; Langdon et al., 2016). Antibiotics cause hypersensitivity and various forms of allergic reactions (Sánchez-Morillas et al., 2010; Lin et al., 2014; Blumenthal et al., 2019). Hence, scientists started to search for new and alternative antimicrobial agents (Chopra et al., 1997; Giglione et al., 2000; Bacha et al., 2016). Hence, keeping in mind the conventional treatment of infections by medicinal plants, modern researchers are using to extract certain natural substances from plants that can prevent growth and/or kill causative pathogens as a way to counter antibiotic resistance (Chanda and Rakholiya, 2011). Studies are being carried out to combat resistance, but this tendency of pathogens to establish resistance seems hard to overcome (Bennett, P.M., 2008). The main concern of the researchers in the recent period is to develop new

ways of manufacturing medicines and ensuring market sustainability (Paters et al., 2010; Golkar et al., 2014; Rai et al., 2014).

Nature has been a source of diverse plants species since ancient times that have acted as a medicinal agent to combat diseases (Jamshidi-Kia, *et al*., 2018). A large number of new medicinal products have been derived from medicinal plants used as traditional medicinal products (Doughari, 2008). A lot of interest and effort has been put forth into studying natural products for producing new antibacterial agents over the last twenty years (Konaté, *et al*., 2012). Traditional medicinal plants and herbs and their various extracts were tested for their antimicrobial properties, and numerous findings showed their effectiveness against various microorganisms (Manandhar, *et al*., *2019)*. As a result, plants may be the building stone for conventional medicine to set new values. This growing interest in investigating indigenous plants can also contribute to the formulation of new therapeutic agents (Satish, 2008). WHO (World Health Organization) reports that in developing and underdeveloped countries, **75%** of the population relies on traditional care, mainly plant-based medicines, to cure primary health disorders (World Health Organization, 2004).

Secondary metabolites from various plants such as flavonoids, alkaloids, and tannins have been shown to have antimicrobial activity (Othman, *et al*., 2019). Similarly, many studies reported lots of herbal extracts having antimicrobial activity (Silva and Fernandes, 2010). Various plant compounds such as alkaloids, peptides, essential oils, phenols, primary and secondary metabolites have medicinal properties that are discovered by successive studies (Bonjar et al., 2004; Lewis, 2006, Doughari, 2008). Plants also generate other therapeutic agents that are effective against bacteria, fungi, and viruses. Most plants have been found to possess the antibacterial activity and are used to treat infections of the urinary and digestive tract, respiratory diseases, and various skin infections (Sibi, 2012). Medicinal and aromatic plants are rich in bioactive compounds. Some of these have antimicrobial and antioxidant effects and also play an essential role in cellular oxidation, as well as being active against pathogens. (Jeruto *et al.*, 2008; Kareru, 2008; Muhammad, 2011). It is, therefore, necessary to characterize the antioxidant and antimicrobial potential of various plants. Several plants derived substances have been found to inhibit the growth of different microorganisms, although the active ingredient in plant extracts is low in concentration but maybe a better source of antimicrobials than synthetic antibiotics (Sibi, 2012). Most plant substances have some functional groups which have several antibacterial mechanisms that are active against microorganisms (Burt, 2004). Therefore, relative to synthetic antibiotics, bacteria are less likely to develop resistance to plant antimicrobials (Ohno, 2003). Research has revealed that berberine inhibits the growth of specific pathogenic bacteria, such as S. *aureus*, *P. aeruginosa*, E. *coli*, and *B. subtilis*.

Spiny sow thistle, Sonchus asper (L.) Hill is possibly a noxious weed originating from the Mediterranean region. It belongs to family Asteraceae and has become a common specie (Cho, *et al*., 2019). This plant has medicinal value and has been used in traditional medicine to cure most human diseases. *S. asper* is commonly used in Pakistan to treat disorders of reproductive dysfunction, oxidative stress, hypertension, renal dysfunction, hormonal disorders, kidney inflammation, hormonal imbalance, impotence, mental disorders, and diabetes (Khan, 2017, Khan et al., 2015, Khan et al., 2013, Khan, 2012a, Khan et al., 2012b, Khan et al., 2012c, Khan et al., 2012d). It is also used as an anti-inflammatory herbal treatment for bronchitis, asthma, wounds, burns, and cough (Wang et al., 2015).

It has been revealed that *S. asper* extracts were used in the treatment of wounds and abscesses and also used as soothing agents (Haemendra, 2013). An investigation to assess the effect of aerial parts

of *S. asper* in induced hypertensive rats utilizing aqueous-methanolic extract of *S. asper* was conducted. There was a substantial ($p < 0.001$) decrease in blood pressure and heart rate in a dose-dependent response (Mushtaq et al., 2016). The efficacy of S. *asper* methanolic extract (SAME) was also tested in albino rats on hormonal dysfunction in thyroid tissue following oxidative stress caused by induced carbon tetrachloride (CCl4). Protective effects of SAME on thyroid hormonal levels, activities of antioxidant enzymes, lipid peroxidation, and DNA damage have been observed, suggesting that SAME can protect thyroid tissue against oxidative damage, possibly by the antioxidant effects of its bioactive compounds (Khan et al., 2012c). The polyphenolic rich methanolic fraction of *S. asper* was used in male rats to investigate its effect on cognitive performance, brain antioxidant activities, and acetylcholinesterase activity. The study emphasized the essential effect of *S. asper* bioactive components on brain function (Khan et al., 2012a). Getting all the capacity for medication *S. Asper* remains an unknown plant, and in some areas, it is considered to be noxious (Haemendra, 2013). This work aimed to recognize antibacterial activities of crude extracts derived from leaves of *S. asper* against multi drug resistant (MDR) human's bacterial pathogens such as *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 43300 and *B. subtilis subsp.* B. spizizinii ATCC 6633.

Materials and Methods

This research work was conducted at the Laboratory of Plant Taxonomy and Microbiology, Quaid-i-Azam University Islamabad, Pakistan. The wild plant selected for this analysis was *Sonchus asper*. It is an annual hermaphrodite herb that belongs to the family of Asteraceae. Leaves of *S. Asper* were collected from Abbaspur village, Poonch district, Azad Kashmir district, Pakistan. Location coordinates are $33^0$49'00.5" N, $73^0$56'39.4E (33.8168080, 73.9442650). In this analysis, leaves of *S. asper* were used as a reference, because the leaves are rich in bioactive constituents.

Further confirmation for the identification of plant specimens was made for their authenticity by a qualified taxonomist from the Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan. For a week, samples were kept under shade to allow them to dry thoroughly, and ground to powdered form and weighed. The dried sample was then subjected to a mere maceration process for extraction. Only 30 grams of powder were taken and soaked separately in 100ml of solvent (methanol, ethanol, and aqua). Such poorly homogenized mixtures were kept in extraction bottles for one week at room temperature (25°C). After one week, mixtures were shaken well, Utilising an electric shaker for 30 minutes. The mixture was filtered twice, and the maximum volume of solvent was removed using ordinary filter paper, and then Whatman filter paper # 1. These solvent extracts were then poured onto a petri dish, covered with a sheet of aluminium foil with multiple pores, and placed for one week in the evaporator. After one week, the acquired extract of *S. asper* was around 0.86, 0.83, 0.88 grams, respectively for each condition.

Four pathogenic bacterial strains were used to assess the antimicrobial potential of these 3 solvents extracts against these bacteria. They were; two Gram-positive, *Staphylococcus aureus* (ATCC43300), *Bacillus subtilis* subspecies B. spizizinii (ATCC6633) and two Gram-negative, *Pseudomonas aeruginosa* (ATCC27853) and *Escherichia coli* (ATCC25922).

A colony from 24 hrs old cultures of these bacteria was taken and mixed in a nutrient broth (Merck #1054430, Dermstadt, Germany) prepared by dissolving 0.13 g of nutrient

broth per 10 ml of distilled water and kept in a shaker incubator for 24 hrs to prepare the inoculum. After 24 hours, the broth was centrifuged at 5000 rpm for 20 minutes from each bacterial inoculum. The supernatant was discarded and the pellets were dissolved in autoclaved saline solution (0.9 g NaC1/100m1 of distilled water) and turbidity was adjusted with a McFarland 0.5 standard solution (corresponding to 1.5 x 10^{-8}colony forming unit per ml at 600nm). Then those inoculums were used to seed the agar plates with nutrients.

The Mueller-Hinton agar (MHA) medium was prepared in 1 litre of distilled water by suspending 38 g/L of nutrient agar (Merck #105450, Dermstadt, Germany); pH was adjusted to 7.0 and autoclaved. It was allowed to cool down up to 45-50°C. Petri dishes were prepared by pouring 30 ml of the nutrient agar to each petri dish and allowed to solidify in the biosafety cabinet. A sterile cotton swab was dipped in the bacterial suspensions and and lawn was prepared on MHA plates by streaking forward and backward. The plates were left for some time so that bacteria on lawn could establish their growth. Small wells, measuring 8 mm, were made of sterile cork-borer. With the use of micropipette, in the respective pre-labelled well, 100 microliters of test solutions were poured in. The standard antibiotic solution as a positive control was prepared by dissolving 2 mg of streptomycin per ml of DMSO. Pure DMSO has been used as a negative control. The plates were incubated at 37°C for 24 hours. After 24 hrs of incubation, the diameter (mm) of clear zones/zones of inhibition were measured around each well corresponding to specific extracts of *S. asper* leaves. All tests were conducted in triplicates. Antibacterial activity of the extracts was determined against all four multi drug resistant bacterial strains, and compared to the inhibition zone produced by standard antibiotics (Streptomycin).

Fourier Transform Infrared Spectrophotometer FTIR:

The Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most effective tools for detecting chemical bonds (functional groups) in compounds. As can be observed in the annotated spectrum, the wavelength of light absorbed is typical of the chemical bond. The chemical bonds of a molecule may be identified by analysing the infrared absorption spectrum. (Nawaz, *et al.,* 2019).

For the FTIR investigation, dried powder of methanolic extracts of plant materials were employed. To make transparent sample discs, 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet. Each plant specimen's powdered sample was put into an FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a scan range of 400 to 4000 cm^{-1}.

Statistical analysis

All of the experiments were conducted in replicates and the data shown in here represent means ± SD. SPSS 21.0 (IBM SPSS Statistics, SPSS Inc., USA). was used for statistical analysis One-way ANOVA followed by Tukey multiple comparison test was employed for finding significant differences ($p<.05$).

Results

The methanolic extract showed (Table 1) significant activity against *S. aureus* ATCC43300 (24mm), *B. subtilis subsp. Spizizinii* ATCC6633 (17mm*), E. coli* ATCC25922 (19mm), and *P. aeruginosa* ATCC27853 (15mm) as compared to streptomycin having a zone of inhibition of 26 mm, 20mm, 20mm, and 18mm for *S. aureus* ATCC43300, *B. subtilis subsp. spizizinii* ATCC6633*, E. coli* ATCC25922*,* and *P. aeruginosa* ATCC27853, respectively. Moreover, no zone of inhibition was shown by DMSO (Figure 1).

Ethanolic extract showed antibacterial activity against *S. aureus* ATCC43300 (19mm), *B. subtilis subsp. Spizizinii* ATCC6633 *(*12mm), *E. coli* ATCC25922 (15mm), and *P. aeruginosa* ATCC27853 (11mm) as compared to streptomycin having a zone of inhibition of 26 mm, 20mm, 20mm, and 18mm for *S. aureus* ATCC43300*, B. subtilis subsp. Spizizinii* ATCC6633*, E. coli* ATCC25922*,* and *P. aeruginosa* ATCC27853, respectively. Moreover, no zone of inhibition is shown by DMSO.

The aqueous extract showed antibacterial activity against *S. aureus* ATCC43300 (12mm), *B. subtilis subsp. Spizizinii* ATCC6633 *(*8mm), *E. coli* ATCC25922 (11mm), and *P. aeruginosa* ATCC27853 (6mm) as compared to streptomycin having a zone of inhibition of 28 mm, 24mm, 13mm and 15mm for *S. aureus* ATCC43300*, B. subtilis subsp. Spizizinii* ATCC6633*, E. coli* ATCC25922*,* and *P. aeruginosa* ATCC27853, respectively. Moreover, no zone of inhibition is shown by DMSO.

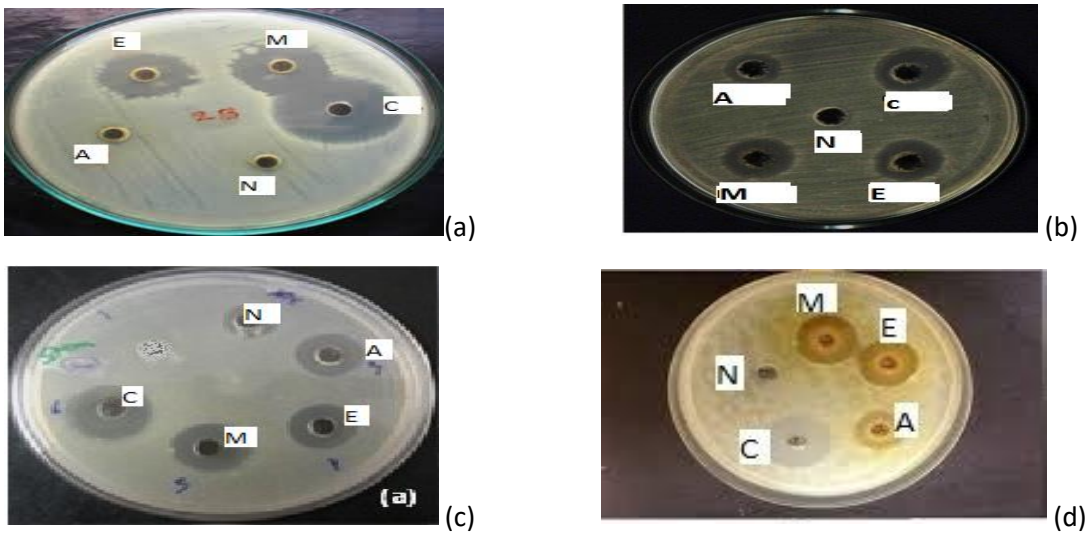

Figure : 1. (a) ***S. aureus*** ATCC43300**,** (b) ***B. subtilis subsp. Spizizinii*** ATCC6633**,** (c) ***E.coli*** ATCC25922**,** (d) ***P. aeruginosa*** ATCC27853.

(M= methanolic extract, E= ethanolic extract, A=aqueous extract, N=negative control and C= positive control)

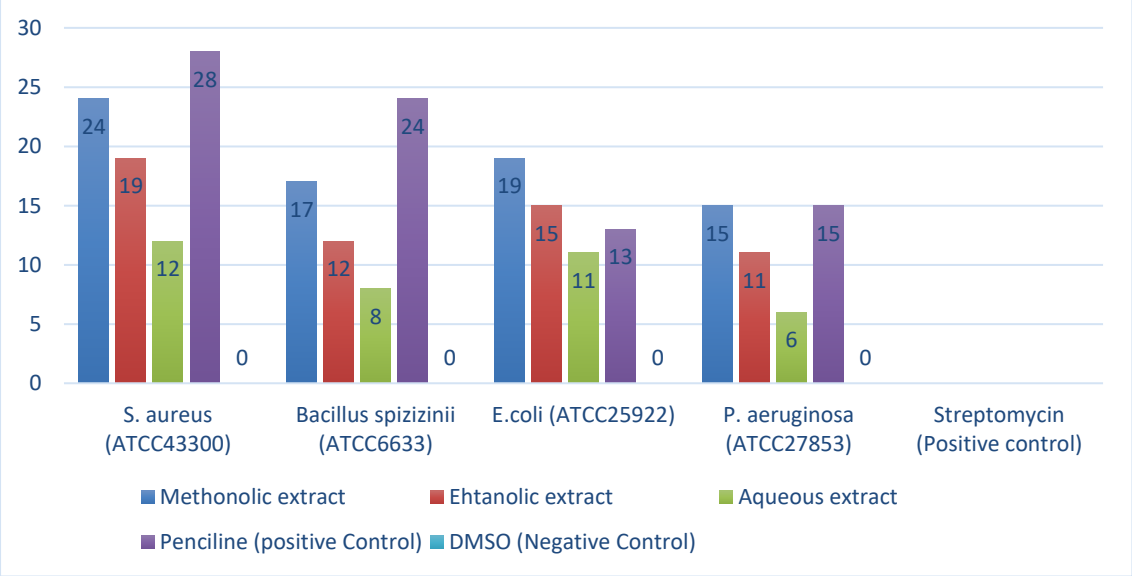

Figure: 2. Graphical representation of comparative antibacterial activity

Table 1: Antibacterial activity of the *Sonchus asper* plant leaves methanolic, ethanolic, aqueous extracts along with positive and negative controls. The zone of inhibitions are shown in mm ± SD for each extract against each multi drug resistant bacterial pathogens.

Bacterial strain	Zone of inhibition (mm) (Methanolic extract)	Zone of inhibition (mm) (Ethanolic extract	Zone of inhibition (mm) (Aqueous extract)	Zone of inhibition (mm) (Streptomycin)
S. aureus (ATCC 2593)	24	19	12	28
Bacillus spizizinii (ATCC 6633)	17	12	8	24
E. coli (ATCC 25922)	19	15	11	13
P. aeruginosa (ATCC 27853)	15	11	6	15

FTIR Analysis:

Absorbance spectra (Figure 3) of leaves methanolic extract showed that *S. asper* is a rich source of organic compounds. Various functional groups indicated by FTIR-spectra showed the presence of organic compounds in *S. asper* extracts like amine, alkane, alkene, alcohols, esters, and ether, sulfoxide, and halo compounds (Table 2).

Componential differences are objectively reflected in spectral differences. Thus, FTIR analysis can validate the existence of the functional component in the given plant extracts, distinguish medicinal materials from sample, and even assess the quality of medicinal materials utilising the FTIR spectrum.

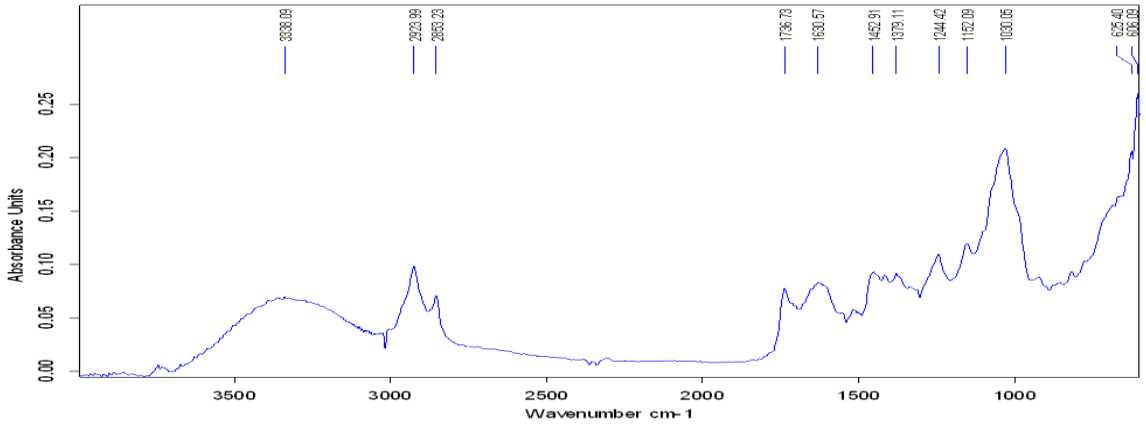

Figure 3. FTIR-Spectrograph

Table 2: FTIR Interpretation of Methanolic extract of *Sonchus Asper*

Sr. No.	Absorbance Frequency	Compounds	Bonding atoms	Absorbance Curve
1	3338.09	Secondary Amine	N-H	Medium
2	2923.99	Alkane	C-H	Medium
3	2853.23	Alkane	C-H	Medium
4	1736.73	Ester	C=O	Strong
5	1630.57	Alkene	C=C	Medium
6	1452.91	Alkane	C-H	Medium
7	1379.11	Alkane	C-H	Medium
8	1244.42	Alkyl aryl ether	C-O	Strong
9	1152.09	Tertiary alcohol	C-O	Strong
10	1030.05	Sulfoxide	S=O	Strong
11	625.40	Halo compounds	C-Br	Strong
12	606.09	Halo compounds	C-Br	Strong

DISCUSSION

This research study was performed to investigate the role of using *S. asper* as a medicinal plant. Given the study by (Brantner, 1994)), the significance of medicinal plants is now well known, especially their use as traditional medicinal products. Pakistan has a large variety of plants that exhibit medicinal importance due to its diverse flora. Pakistan has a wealth of medicinal plant resources, 12% of which have been registered and recognized as carrying medicinal values, and marginal communities have used them to cure various diseases (Shinwari, 2010).

Since ancient times *Sonchus asper* has been used as ethnomedicine to treat various ailments. The therapeutic benefit that different plants possess is a contribution of their antimicrobial properties. The purpose of the present research was to determine the biological activity of *S. Asper* plant of family Asteraceae. It was found that the crude extracts of *S. Asper* had exhibited significantly ($p<0.05$) higher antibacterial activities prepared from the plant leaves against *S. aureus* ATCC43300 and *E. Coli* ATCC25922. The results of this analysis showed that the methanolic extract of *S. Asper* has considerable potential to inhibit growth bacteria, and this claim is also supported by (Chandrasekaran, 2004)), which confirmed that methanol could be used as the most efficient solvent for extraction of antimicrobial compounds from plants.

There is currently scarce evidence available on the antimicrobial efficacy possessed by Astreacea family plant extracts. A study reported that methonolic extracts of *Centaurium erythraea* (MIC=1.0 mg/ml) and *Prunus padus* (MIC=0.01 mg/ml) against methicillin resistant *Staphylococcus aureus showed promising activity.* (Kumarasamya , 2002). The extracts under examination were from the plant's aerial parts (leaves), and results concide with above mentioned study as methanolic extract showed activity against *S. aureus*.

Another study (Sengul, 2009) described the zone of inhibitions by methanolic and aqueous extracts (300 mg/ml) from the aerial parts of *Taraxacum officinale* against thirty-two particular food-borne microorganisms. Whereas, the most prominent antimicrobial activity for the methanolic extract of *T. officinale* was observed against 11 strains of B. *cereus*, *E. coli* and *S. aureus*. That antimicrobial activity

was then linked to the extract's organic content. The methanolic extract of *S. asper* in the present analysis showed potent inhibitory activity against *S. aureus* ATCC43300**,** *B. subtilis subsp. Spizizinii* ATCC6633**,** *E.coli* ATCC25922**,** *P. aeruginosa* ATCC27853. Although ethanolic and aqueous extract showed some activity too but not significantly higher than methanolic extracts activity.

Agar well diffusion and methods of spreading discs are usually used to screen antimicrobial or precisely antibacterial activities. This research also carried out with the adoption of this agar well diffusion technique to establish the antimicrobial activity of methanolic, ethanolic, and aqueous extracts of this particular plant (*S. asper*). Some other workers, like (Victor, 1996), used a similar technique. For this investigation, Mueller Hinton Agar was used to cultivate bacteria. Microbiologists have used this widely for the production of bacterial pathogens. This medium is known to be sufficiently competent to allow the growth of all the pathogens used in this research. The richly enriched extract inhibits bacterial growth more effectively than the less enriched one (Panthi, 2006).

Our results indicated that crude extract from *S. asper* leaves showed antibacterial activity at a concentration of at 15mg/ml. Antibacterial activity of plant extracts has also been found to be concentration dependent (Othman, *et al*., 2011). The richly enriched extract more effectively prevents bacterial growth than the less enriched one (Panthi, 2006). Some plants' methanolic extract showed marked antibacterial activity against *E. coli, B. subtilis K. pneumoniae,* and *P. aeruginosa* (Kelmanson, *et al*., 2000). In categorizing antibacterial activity against Gram-positive and Gram-negative bacteria, a much higher number of plant extracts will be predicted to be effective against gram-positive bacteria than gram-negative (Panthi, 2006; Padhi, 2011). Also, in support of this view are our findings as methanolic extract of *S. asper* showed effective Gram-positive bacteria (*S. aureus* ATCC43300 and *B. subtilis subsp. Spizizinii* ATCC6633) inhibitory activity.

Findings from this study showed that leaves extract of medicinal plant such as *S. asper* exhibit antibacterial activity. Similar results were described by (Narod, 2004) as the hexane, methanolic and aqueous extracts of leaf and stem of *Toddalia asiatica* showed significant antibacterial activity against both Gram-negative and Gram-positive bacteria.

There are a number of reasons why rural people sometimes use plants as medicines. Because of health improvement after herbal healing, herbal medicines are less costly, conventional drugs are not available even in rural areas, whereas available drugs are either fake or obsolete, and people are often more used to natural healing and happy with it (Padhi, 2011). This screening showed that crude extracts of *S. asper* displayed a wide range of antibacterial activity against both Gram-positive and Gram-negative bacteria.

The present research study used FTIR spectroscopy of methanolic extract and found that there are a variety of essential organic compounds in *S. asper* like, Amine, Alkane, Alkene, Alcohols, Esters, and Ether, sulfoxide, and halo compounds. The findings of this research corroborated earlier discoveries made by different plant biologists and taxonomists.

Many scientists used the FTIR spectrum to differentiate between closely related plants and other creatures. The findings of this research led to the development of a new phytochemical marker for identifying medicinally significant plants. The structural elucidation and identification of active components found in Sonchus asper leaves will need more sophisticated spectroscopic investigations. It is also confirmed by the FTIR study of *Bauhinia racemosa*'s methanolic leaf extracts, which demonstrated the existence of phenolic compounds and carbohydrates as major functional groups

(Gaurav, 2008). The presence of Amine, Alkane, Alkene, Alcohols, Esters, and Ether, sulfoxide, and halo compounds in *S. asper* as shown by FTIR analysis is also supported by the study of (Ragavendran, 2011) which examined the functional groups of carboxylic acids, amines, amides, organic hydrocarbons and halogens contributing to the various therapeutic properties of *Aerva lanata*.

CONCLUSION

The methanolic crude extracts prepared from *S. asper* leaves showed more significant antibacterial activity against multi drug resistant bacterial pathogens. It showed strong antibacterial activity against *S. aures* ATCC43300. These data convey that the plant extracts have great potential against multi drug resistant microorganisms and can be used as antimicrobial compounds. Therefore, it can be said that compounds of *S. asper* can be useful in treating pathogen-caused infections as well as pathogenic resistant bacteria after clinical confirmation and molecular investigations.

Also, from the FTIR results shown by the methanolic extract of *S. asper*, it can be concluded that the leaf extract of *S. asper* with its phytoconstituents may serve as antibiotics. The present study has also revealed the importance of natural products in controlling antibiotic-resistant bacteria, which pose a threat to human health and can serve as an essential platform for the development of alternative, inexpensive, safe, and effective medicines.

Authors Contributions: Rizwana Kausar: Main researcher and Author

Lubna Razzaq: contributed in reviewing of literature

Faiz-ur-Rahman: Contributed FTIR interpretation and analysis

Dr. Muhammad Zafar: Supervised this research

Alyaa Abdulhussein Alsaedi: contributed FTIR experiment

Conflict of Interest: The authors declare that they have no conflict of interest.

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