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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL PROPERTY OF OPUNTIA FICUS INDICA SEED EXTRACT AGAINST WOUND INFECTING BACTERIA

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ABSTRACT
Opuntia ficus-indica, also known as Indian fig or prickly pear, is a cactus known for its therapeutic and nutritional properties. Its unique flavor and texture, along with potential health benefits, are attributed to its compounds found in its tissues. This article explores the plant's phytochemical makeup, antioxidant capabilities, and antibacterial activities. It highlights the plant's antibacterial, anti-inflammatory, and antioxidant properties, and its distinctive red and yellow coloration is attributed to the presence of betalains. Opuntia ficus-indica, a plant with strong antioxidant properties, has been found to be beneficial in reducing the risk of chronic diseases. Its phenolic chemicals and betalains protect cells from harmful free radicals, promoting overall health. Additionally, extracts from Opuntia ficus indica can promote the body's ability to produce superoxide dismutase and catalase, a couple of antioxidant enzymes. Opuntia ficus-indica seed extracts have shown significant antibacterial activity against various bacteria causing wound infections. Bioactive substances like alkaloids and flavonoids contribute to their antibacterial activity. Research suggests these extracts may be useful against both Gram-positive and Gram-negative bacteria, making them a viable natural alternative for treating bacterial infections due to their broad-spectrum antibacterial activity.

Keywords: Prickly Pear, Betalains, Betacyanins, Superoxide Dismutase, Wound Infecting Bacteria.

INTRODUCTION
The cactus (Opuntia ficus-indica), genus belonging to the Cactaceae family, commonly referred to as the prickly pear. According to (Pareek OP et al.,2003), Opuntia ficus indica produces sweet, rich nutrients, edible fruits and soft cladodes that are used in fresh green salads and vegetables. Approximately 130 genera and nearly 1500 species are said to make up the family Cactaceae, It is native to various regions around the world, such as the Near East, Australia, South Africa, India, and the Mediterranean basin. They are all excellently suited to a range of the temperatures and desert terrain. Although in some countries different portions of the plant are used in the food and cosmetic industries, this species is additionally Grown for its prickly pear, a nutritious fruit, in South Africa, Mediterranean regions, and South America (Griffith MP et al., 2004). Opuntia ficus-indica fruits and stems have historically been implemented for a variety of therapeutic purposes in folk medicine throughout several nations (Hunt D. and Taylor et al., 2006). However, many scientists have been focusing their efforts on studying the genus Opuntia to determine the characteristics of plants that might serve as the basis for their use in the treatment and prevention of chronic diseases. Due to the growing

awareness that many individuals use this plant for self-medication, The effectiveness and safety of the phytochemicals in the genus Opuntia have drawn more attention in the past few years from the clinical pharmaceutical community.

Glycosylated flavanols, dihydroflavonols, flavanones, and flavonoids are present in cactaceae plants and fruits (Kuti et al., 1992). Multiple research investigations have discovered a substantial correlation between the antibacterial and antioxidant capabilities of extractable polyphenol extracts from Opuntia spp. and their phenol concentration. Most researchers have also discovered that the extraction solvents and processing techniques used can change the yield, phenolic component profile, and biological activity of the extracts under investigation (Abou-Elella and Ali et al., 2014). Opuntia fruits contain a variety of bioactive substances, including flavonoids and phenolic compounds, which are antioxidant components.

Natural antioxidants may be found in plants. To combat reactive oxygen species (ROS), it makes a variety of antioxidant molecules.ROS are many forms of activated oxygen, such as free radical species, hydroxyl radicals (-OH), superoxide anion and non free radicals like H2O2 and single oxygen (Ameer et al., 2022; Kang et al., 2022). These chemicals exacerbate cellular damage and the aging process. Bioactive substances like quercetin, isorhamnetin, and kaempferol are found in cactus pears.

According to (Nenah et al.,2013), these bioactive chemicals exhibit potent antibacterial effects on a variety of microorganisms, such as Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. Opuntia Ficus indica has considerable antibacterial action against Vibrio cholera in its aqueous, ethanolic, and methanolic extracts (Sánchez et al., 2010). According to (Belay et al., 2015), S. marcescens, E. coli, and S. thermophilus are only a few of the microorganisms that the ether and alcoholic extracts of cactus pears effectively kill. Cactus pear extracts were investigated for their antibacterial efficacy against bacteria that cause wound infections and are drug-resistant, including Enterococcus faecalis, E. Coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae. Through the Food and Agriculture Organization (FAO) of the United Nations (UN), Opuntia ficus indica has progressively come to be economically significant in agriculture and the larger international community. The current study's objectives were determining the minimum inhibitory concentration (MIC) for various microorganisms, the antioxidant ability of Opuntia ficus indica seed extract using the DPPH assay, and the phytochemical compositions and polyphenolic contents of the extract.

2.MATERIALS METHOD

Collection of plant material

Fresh fruit of Opuntia ficus indica was collected from a dry place. The seed was cleaned properly and shadow dried. After complete drying, the seed was separated and powdered. It was stored in an airtight container.

Extraction of seed

A mixture of 250 ml distilled water, ethanol, and 25 grams of finely powdered seed was used. After that, the suspension is allowed to incubate for 48 hours, or roughly two days, with periodic shaking. The contents are filtered via Whatman No. 1 filter paper following a 48-hour incubation period. The filtrate was then subjected to analysis.

2.1 Qualitative Phytochemical Screening Phytochemical Analysis

The plant extract solutions were assessed for the existence of the phytochemical analysis by using the following standard methods.

2.1.1 Test for proteins Millon's test:

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test:

Crude extract when boiled with 2 ml of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence of amino acids and proteins.

2.1.2 Test for carbohydrates Fehling's test:

Fehling's test involves mixing equal volumes of Fehling A and Fehling B reagents, adding 2 ml to crude extract, and gently boiling it. The presence of reducing sugars is indicated by the appearance of a brick-red precipitate at the bottom of the test tube.

Benedict's test:

As 2 ml of Benedict's reagent was added to crude extract and heated, a reddish-brown precipitate developed, signifying the presence of carbohydrates.

Molisch's test:

2ml of Molisch's reagent were combined with crude extract, and the mixture was thoroughly shaken. Next, a cautious 2 ml of concentrated H_2SO_4 was gently poured along the test tube's side. The presence of carbohydrates was suggested by the appearance of a violet ring during the interphase.

Iodine test:

There was a mixture of 2 ml of iodine solution and crude extract. The presence of the carbohydrate was identified by a dark blue or purple coloring.

2.1.3 Test for phenols and tannins

Crude extract was combined with 2ml of a 2% $FeCl_3$ solution. Phenols and tannins were characterized by a blue-green or black coloring.

2.1.4Test for flavonoids Shinoda test:

Concentrated HCl and a few pieces of magnesium ribbon were combined with crude extract was added drop wise. Pink, scarlet color appeared after a few Crude extract was mixed minutes which indicated the presence of flavonoids.

Alkaline reagent test:

2ml of a 2% NaOH solution was used to cure. When a few drops of diluted acid were added, the bright yellow color that had formed went colorless, indicating the presence of flavonoids.

2.1.5 Test for saponins

In a test tube, 5 ml of distilled water was combined with crude extract, and the mixture was agitated vigorously. It was believed that the production of stable foam indicated the presence of saponins.

2.1.6 Test for glycosides Liebermann's test:

2ml of acetic acid and chloroform were combined with the crude extract. Ice was used to chill the concoction. A precise concentration of H_2SO_4 was introduced. When the hue changed from violet to blue to green, it meant that the glycone part of the glycoside, or the steroidal nucleus, was present.

Salkowski's test:

Chloroform (2 ml) was combined with crude extract. After that, 2 ml of concentrated H2SO4 was added and given a gentle shake. The existence of a steroidal ring, or the glycone portion of the glycoside, was indicated by a reddish-brown colour.

Keller-Kilani test:

Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2 ml of concentrated H2SO4.The presence of cardiac glycosides was indicated by a brown ring at the interphase.

2.1.7 Test for steroid

Additions of concentrated H2SO4 were made after the crude extract and 2 ml of chloroform were combined. The presence of steroids was identified by a red hue formed in the bottom chloroform layer. Another experiment involved combining 2 milliliters of chloroform with crude extract. Next, the mixture was added to 2 milliliters of concentrated H2SO4 and acetic acid, respectively. Steroid presence was revealed by the greenish color was developed.

2.1.8 Test for terpenoids

After dissolving the crude extract in 2ml of chloroform, it was dried out. After adding 2 ml of concentrated H2SO4, this was boiled for approximately 20 minutes. Terpenoids were indicated by a grayish tint.

2.1.9 Test for alkaloids

2ml of 1% HCl was combined with crude extract and heated gradually. The mixture was then supplemented with Wagner's and Mayer's reagents. The ensuing precipitate's turbidity was seen as proof that alkaloids were present.

2.2. In Vitro Antioxidant Activity

Assay of antioxidant activity by DPPH (1, 1-diphenyl-2- picrylhydrazyl) free radical scavenging activity

DPPH was used to find out how well the Opuntia ficus indica seed extract got rid of free radicals. (Molyneux, P et al.,2004). The DPPH solution (0.006% w/v) was prepared in methanol. Different concentrations of the ethanolic and aqueous extract of Opuntia ficus indica seed (20, 40, 60, 80, and 100 µg/ml) were prepared. 3 ml of different concentrations of the seed extract of Opuntia ficus indica were mixed in the dark with 300 µl of DPPH solution. Ascorbic acid is an effective antioxidizing agent, is taken as a standard and prepared in different concentrations using methanol (20, 40, 60, 80, and 100 µg/ml). 3 ml of different concentrations of a standard solution of ascorbic acid were mixed with 300 µl of DPPH solution in the dark. The prepared solution of ascorbic acid and seed extract samples was incubated for 30 minutes, and then, at 517 nm, absorbance was measured with a UV spectrophotometer. The experiment is expressed as the sample's inhibition percentage of free radicals, with methanol acting as a blank. This was computed using the following formula:

DPPH radical scavenging activity (%) = (Control OD-Sample OD) / Control OD x 100

2.3 Antimicrobial Activity Principle

On a freshly seeded plate, the test organisms interact with the antimicrobials found in the seed extract as they diffuse out into the medium. There will be a confluent lawn of growth, resulting in uniformly round zones of inhibition. Millimeters can be used to measure the diameter of the zone of inhibition.

Reagents

1. Mueller Hinton Agar Medium

The medium was prepared by dissolving 33.9 g of the commercially available Mueller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs. pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm Petri dishes, while still molten (25–30 ml/plate).

Nutrient broth

One liter of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (Hi Media) in 1000 ml of distilled water and boiled to dissolve the medium completely. After the medium was filled to the correct amount, it was autoclaved for 15 minutes at 121ºC (15 pounds of pressure).

Procedure

Bacterial strains were cultured for twenty-four hours on petri dishes that held twenty ml of Muller-Hinton medium. 20µl of the plant extracts were added after the wells were cut. After that, the plates were incubated for 24 hours at 37°C. The diameter of the inhibition zone that developed around the well served as an indicator of the antibacterial activity.

RESULTS

Table 1: PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT AND ETHANOL EXTRACT OF Opuntia Ficus indica SEED EXTRACT

S.NO	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	Carbohydrate	+	+
2	Tannins	+	+
3	Saponins	+	-
4	Alkaloids	+	+
5	Flavonoids	+	+
6	Glycosides	+	+
7	Quinones	+	+
8	Phenols	-	+
9	Terpenoids	+	+
10	Steroids	+	+

(Symbol (+) indicates positive and (-) indicates negative)

QUALITATIVE PHYTOCHEMICAL ANALYSISOF AQUEOUS AND THANOLIC EXTRACTION OF OPUNTIA FICUS INDICA SEED EXTRACT

A qualitative phytochemical analysiswas carried out on the crude extract of aqueous and ethanolic extracts of Opuntia ficus indica seed extract to determine severalphytochemicals such as steroids, quinones, phenols, alkaloids, flavonoids, glycosides, tannins, saponins, and carbohydrates. Table 1 shows the results of the study. Phytochemicals such as polysaccharides, tannins, saponins, alkaloids, flavonoids, glycosides, quinones, and steroids are present in the aqueous extract. On the other hand, the ethanolic extraction consist of phytochemicals includes steroids, quinones, phenols, alkaloids, flavonoids, glycosides, tannins, and terpenoids. Saponins were not present in the ethanolic seed extraction (Romero-Orejon et al., 2022). Based on the testing and findings in the aqueous and ethanolic extracts of prickly pear cactus seed extraction studied, both contain the same number of phytochemicals. This qualitative phytochemical testing shows that the seed extract contains many important medicinal phytochemicals.

PHYTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF OPUNTIA FICUS INDICA SEEN EXTRACT

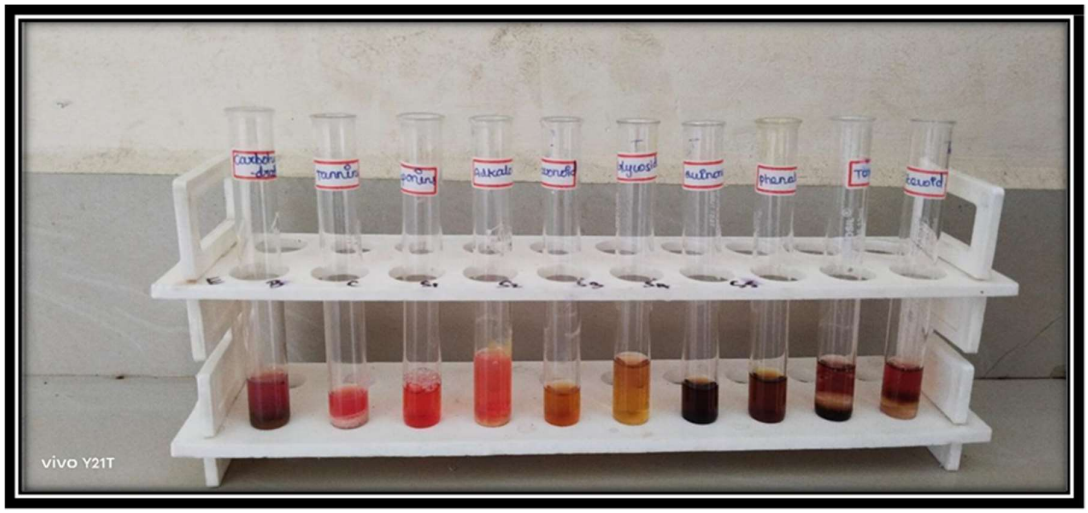


FIGURE: 1 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQUEOUS SEED EXTRACT

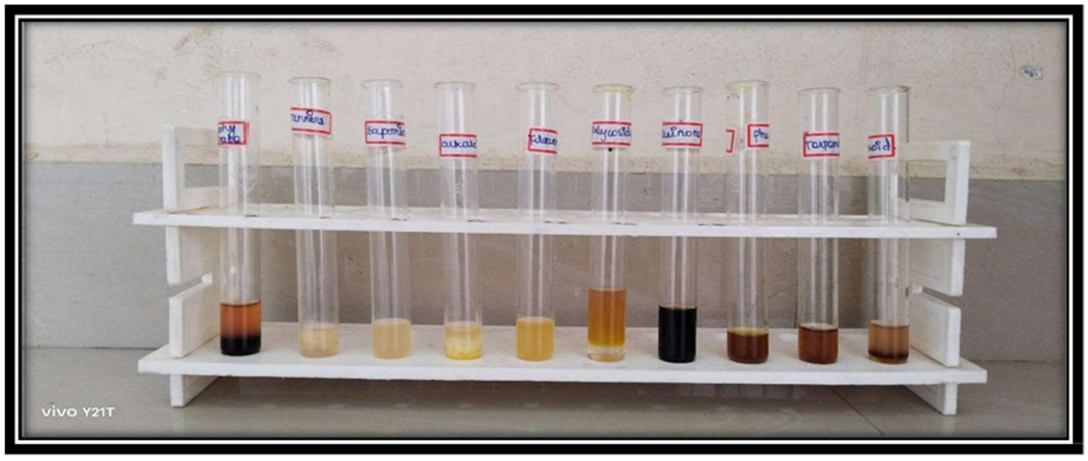
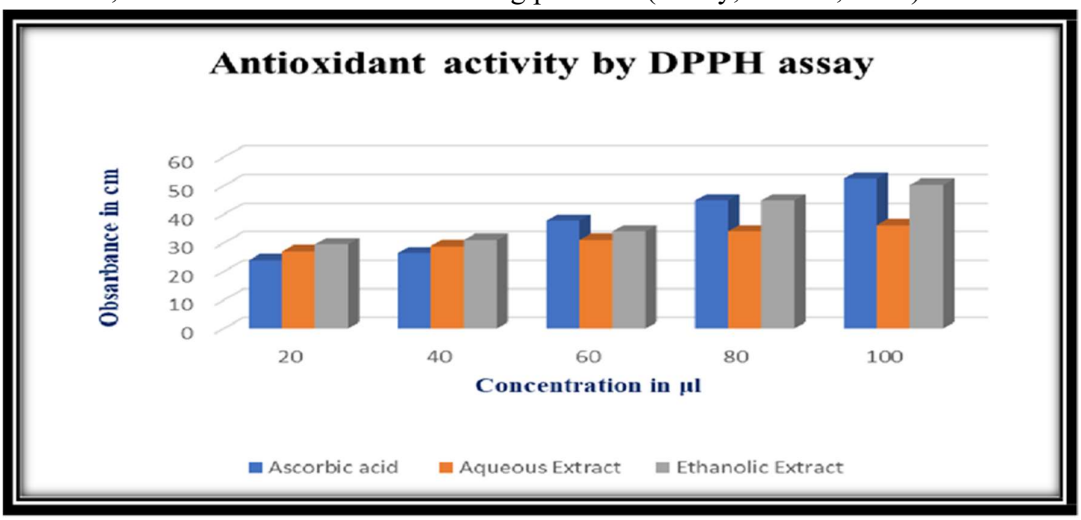


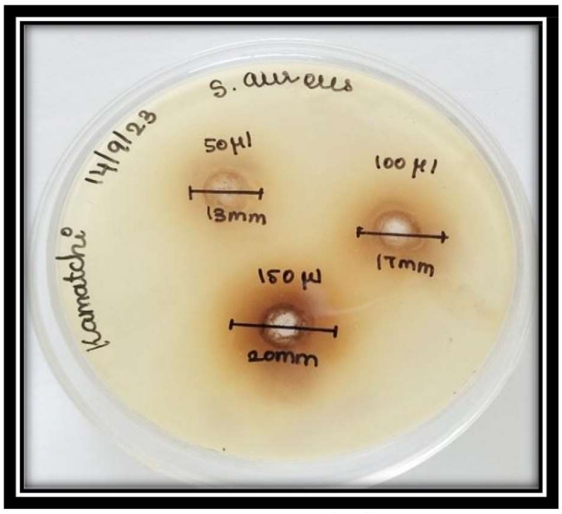
FIGURE: 2 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOLIC SEED EXTRACT

ANTIOXIDANT ACTIVITY

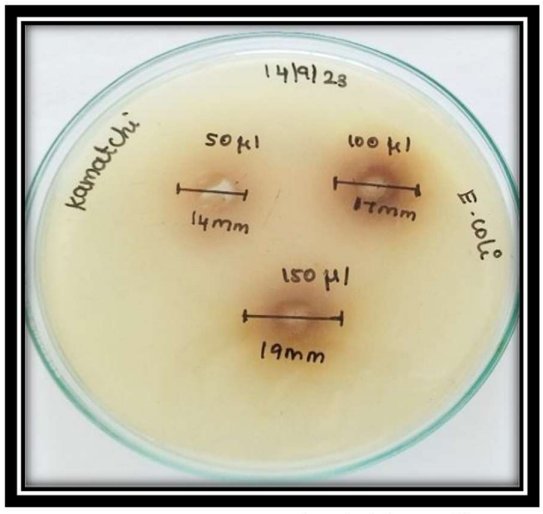
The antioxidant activity of Opuntia ficus Indica seed extract was analyzed by the DPPH radical scavenging assay. For this activity, aqueous and ethanolic crude extracts were taken. Both aqueous and ethanolic seed extracts have free radical-scavenging potential. Compared to aqueous extract, ethanolic extract has high DPPH radical scavenging potential. 20 and 40 µg of aqueous and ethanolic extract have higher activity compared to ascorbic acid, and other concentrations like 60, 80, and 100 µg have lower activity than ascorbic acid. Ethanolic extract has almost the same level of activity as ascorbic acid. An increased concentration of Opuntia Ficus indica seed extract (aqueous and ethanolic extract) and ascorbic acid shows increasing absorbance, which has a free radical-reducing potential (Ozsoy, N et al., 2008) .



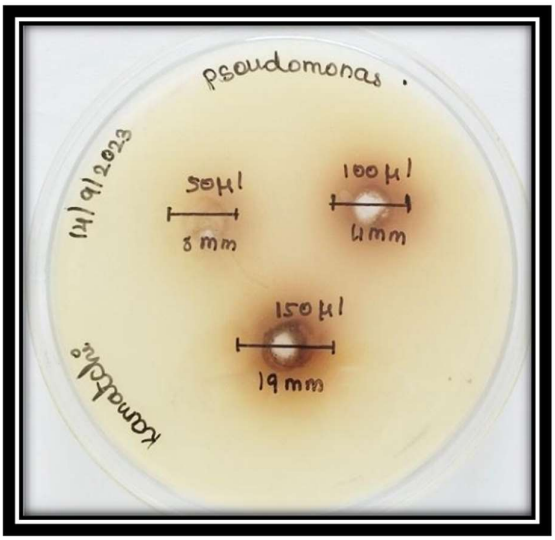
ANTIBACTERIAL ACTIVITY AQUEOUS EXTRACT



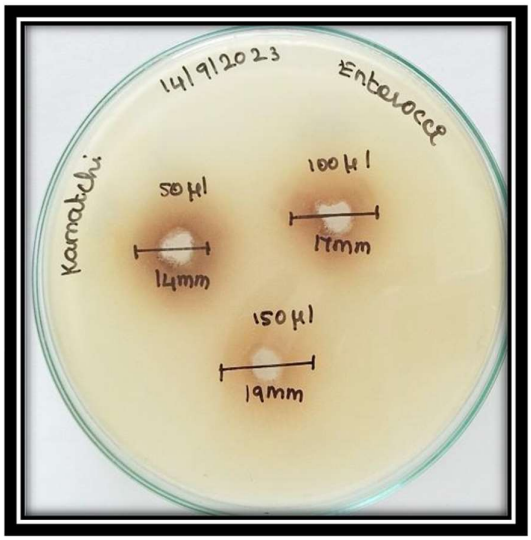
Staphylococcus aureus



Escherichia coli



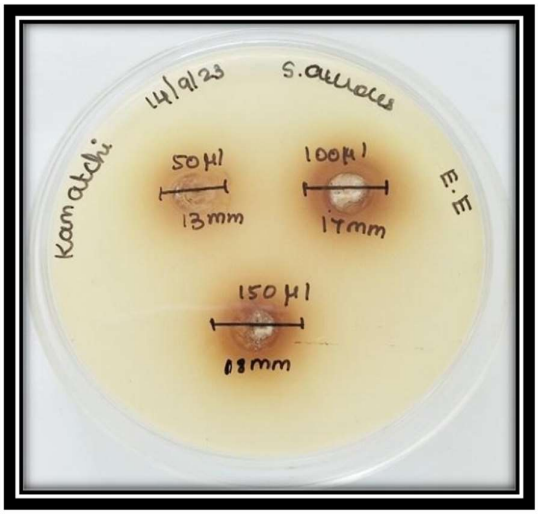
Pseudomonas



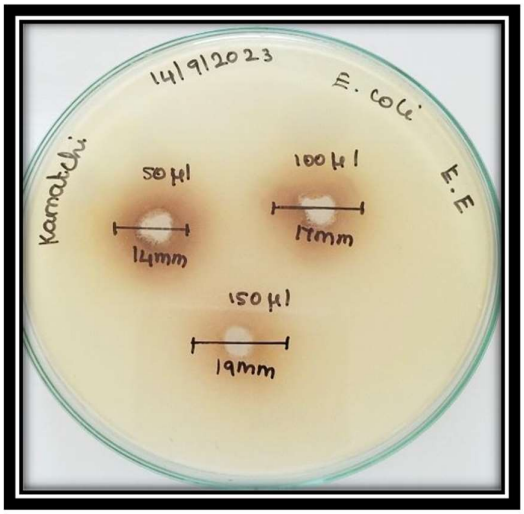
Enterococci

Antibacterial activity of aqueous seed extract *opuntia ficus indica*

ETHANOLIC EXTRACT



Staphylococcus aureus



Escherichia coli

Pseudomonas Enterococci

Antibacterial activity of ethanolic seed extract opuntia ficus indica

The antibacterial activity of aqueous and ethanolic extractions of the Opuntia ficus indica seed extract against wound-infecting bacteria was analyzed by the agar-well diffusion method. This antibacterial activity is done by four bacterial strains: two-gram positive bacteria and two-gram negative bacteria, namely Staphylococcus aureus, Enterococcus, pseudomonas, and Escherichia coli. These are all the bacteria that may cause wounds to living organisms. These wounds infecting bacterial growth were inhibited by using prickly pear cactus seed extract at different concentrations (50, 100, and 150µl) of aqueous and ethanolic extraction of seed extract. Both aqueous and ethanolic

extracts show a zone of inhibition. Gentamicin and Ampicillin serve as a positive control and DMSO taken as a negative control. Compared with aqueous extract, the ethanolic extraction shows slightly increased or higher in the zone of inhibition with the higher concentration. Even though the inhibition zone of positive control shows a larger than all the three different concentrations of the seed extract. The major wound infecting bacteria is staphylococcus aureus. It shows the increasing zone of inhibition at the high concentration(150µl) (Ali et al., 2022 &S.K.; Mahmoud et al., 2022)

CONCLUSION

Phytochemical and various biochemical activity such as antioxidant activity and antibacterial activity of aqueous and ethanolic extract of opuntia ficus Indica seed extract were determined based on the results obtained through in vitro methods. In antibacterial activity different concentrations (50, 100 and 150 µg/ml) of aqueous and ethanolic extract of Opuntia ficus indica seed extract were tested against wound infecting bacterial strain and it exhibited good antibacterial activity. At high concentration of seed extra can inhibit wound infecting bacterial growth. In antioxidant activity of seed extract which shows significant activity at DPPH assay. This study, phytochemical and biochemical analysis of opuntia ficus indica seed extract provide some suspected pharmacological activity

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