Preliminary Study of the Phytochemical analysis, Antimicrobial, Antioxidant and Cytotoxic activity of *Cirsium swaticum*

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Abstract: The current research work examines the phytochemical profile, antibacterial, cytotoxic, and antioxidant properties of Cirsium swaticum crude extract. The findings demonstrated the presence of numerous significant phytochemicals in Cirsium swaticum, including carbohydrates, cardiac glycosides, saponins, flavonoids, phenol, Curamins, oils, and lipids. Five different bacterial strains i.e-(*Proteus mirabilis, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Salmonella typhi, and E. coli)* were tested for antibacterial activity using the agar-well diffusion method. The obtained results demonstrated highly substantial anti-bacterial action, and among the several extracts, the methanolic extract from the leaves is superior to that from the stem and root against our tested bacterial stains. The extract was also evaluated for antifungal activity and the results showed that it was highly effective against five different fungal strains, including such as (*Alternaria species, Carvolaria species, Fusarium species, Helminthosporium specie* and *Rhizopus species)*. This is the first report on the pharmacognostic studies of Cirsium swaticum Petr and is useful in the characterization of the crude drug. Earlier, the other species of the genus Cirsium swaticum were screened and found potential antimicrobial activities, but Cirsium swaticum Petr was not previously studied. Cirsium swaticum Petr was tested for its ability to scavenge free radicals by inhibiting them in different concentrations such as 1, 3, and 5 mg/ml using the conventional DPPH assay. The findings demonstrate that the crude methanolic extract of Cirsium swaticum Petr at concentration 1 mg/ml shows the maximum percent inhibition (70%), while the smallest percent inhibition was obtained at concentration 5 mg/ml (19%). The larvae of brine shrimp were used to test the cytotoxicity. The results showed that when the Cirsium swaticum methanolic extract was tested at different doses, i.e. 100, 500, and 1000 ug/ml, the maximum percentage of deaths was recorded at 1000 ug/ml (90%), the maximum percentage of deaths was

recorded at 500 ug/ml (60%), and the minimum percentage of deaths was recorded at 1000 ug/ml (30%).

Keywords: Phytochemicals, Antimicrobial antioxidant and Cytotoxic activity of Cirsium *swaticum* Petr.

Introduction

There are more than 32,000 recognised species of flowering plants in the family Asteraceae, sometimes known as Compositae, which belong to the order Asterales. Compositae, also known as the aster, daisy, composite, or sunflower family, were originally identified in 1740. Only the Orchidaceae has more species than the Asteraceae, and it is uncertain whether family has more species overall because we don't know how many species are still alive in each family [1]. The genera Mimulus, Penstemon, Digitalis, Veronica, and Verbascum are all relatively significant members of the family [2]. High amounts of saponins and flavonoids found in plants of the genus Verbascum are employed mostly in folk medicine as anaesthetic healing agents for burns and tumours, as well as for cough and eye inflammation [3]. The leaves and blossoms of select Verbascum species are used in Turkish medicine to treat a variety of respiratory conditions, including cough, bronchitis, asthma, and tuberculosis. Additionally, it has been claimed that certain Verbascum species are effective in the treatment of haemorrhoids, discomfort, fungus infections, diarrhoea, and wound healing. They also reportedly exhibit inhibitory activity against the influenza viruses B and A2 and murine lymphocytic leukaemia. [4]. The floral oil is mostly used to treat skin conditions, while it can also be used to relieve earaches and external eczema. Additionally, it was noted that the Verbascum species is used as a tea to treat abdominal pain, a light sedative, and urinary tract infections [5-7]. To treat stomachaches, they are typically drunk as a tea. The demand for innovative antibiotic prototypes has arisen as a result of the ever-growing resistance of human diseases brought on by microbes. A recent study has revealed that higher plants are a source of antibiotics. Numerous plants in the genus Verbascum, including Verbascum Thapsus, have been employed for antibacterial purposes [8]. Although plants are a major source of novel medications, the promise of higher plants has not yet been fully realised. There are between 250,000 and 500,000 species of plants, but only a limited number have been examined phytochemically, and our understanding of biology and pharmacology is inadequate. The physicochemical properties inform us of the drug's inorganic composition, soluble components, and purity. Therefore, in the herbal industries,

physicochemical parameters are used to monitor drug purity, minimise the possibility of adulteration, and also aid to raise the standard of medications. [9]. Bacteria are identified as the number one common microorganisms that produce a variety of hazardous diseases linked to HIV/AIDS. Microorganisms create a variety of ailments [10]. The germs that cause disease in humans have been linked to morbidity and mortality. Numerous antibacterial medications were created by pharmaceutical corporations, but the rate of bacterial resistance to these medications also rose, becoming a concern on a global scale [11]. According to observations, the double membrane is the primary contributor to antibiotic resistance in gram-negative bacteria [12]. Research on antimicrobial substances was conducted on the recently discovered plant Verbascum arianthum to examine the antibacterial and antifungal activities of the specie. It was established by literature review that plants are a major source for antimicrobial medications [12].

Fig 1: ***Cirsium swaticum*** **petr.**

2. Materials and Methods

The *Cirsium swaticum* Petr. fresh leaves, bark, and root were harvested in Pakistan's District Kohistan. The collection area's coordinates are 34.5482° N and 73.3532° E. They were identified by Prof. Dr. Manzoor Hussian, former Dean and chairman of the Department of Botany Hazara University Mansehra, Pakistan. After identification the specimen were deposited in the Herbarium of Hazara University (HUP) for permanent record. The plant materials were washed with tap water separated and dried in shade for 15 days. These materials were used afterward for phyto-chemical, and biological activities of *in-vitro* biological activities

2.1. Phytochemical analysis

The crude extracts were examined for the presence of various compounds and ethanol, methanol and distilled water were utilised as the solvents. In a mixture of 1:10 ethanol, methanol, and distilled water, the leaves, root and stem were prepared. Five grammes of powdered medication were then soaked in 50 ml of the aforementioned solvents and let to soak for three to four days while being stirred often each day. The filtrate was then utilised for various phytochemical studies such as Sapon, flavonoids, quinine, tannins, carbohydrate, alkaloids, glycoside, terpenoids, phenols, cumarins, pholobutannins, authroquinones, and changed oil and lipids. The filtrate was then passed through Wattman's filter paper [13].

2.2. Anti-bacterial activity

Preparation of plant extracts

A grinder was used to reduce the dried plant material to a fine powder. Then, 10 grammes of powder from each plant part were mixed with 450ml of 80 percent (80%) methanol and the mixture was shaken constantly for three days. The solvent from the third day which had been incubating for 48 hours was filtered using filter paper. Methanol was evaporated using a rotating evaporator at 40°C after the filtration process and the vacuum pressure was maintained as it decreased. Methanol was successfully extracted from plant extract in this manner and pure extract was produced for additional research. Plant extract also known as crude methanolic extract was stored at room temperature. The agar well diffusion method was used to test the antibacterial activity of plant crude extract [14].

Media used for growth of bacteria

a) Nutrient Broth media

(NB) was made by combining 0.16 grammes of NB media with 20 millilitres of distilled water for the growth of bacteria, and it was then autoclaved at 121 degrees Celsius for 20 minutes to sterilise it. The pH was set to be 7.

b) Nutrient agar media

The nutrient agar media needed to perform antibacterial activity was made by combining 5.04 grammes of NA (28 grammes per litre) medium in 180 ml of distilled water. It was then autoclaved at 121 degrees Celsius for 20 minutes to sterilise it. The pH was set to be 7.

Sub culturing:

In this study, four bacterial strains—PA, ST, EC and MRSA—were employed. Slants and medium containing nutrient broth was used to subculture the microorganisms. For the purpose of creating slants, 7.56 gramme of NA was dissolved in 160 ml of distilled water and sterilized in an autoclave for 20 minutes at 121 OC. After allowing sterilized media to cool for an hour, each test tube received a different batch of media. For the purpose of growing plants, test tubes were covered with cotton plugs and kept at room temperature. Each test tube contained 5ml of medium; generally four test tubes were used for four bacterial strains. A wire loop was used to transfer bacteria into each test tube once the agar had fully solidified. In order to transfer the bacteria into test tubes containing nutrient broth, the sterilized loop was touched on a bacterial culture and picked up the bacteria. These test tubes were stored in an incubator, and the loops were sterilized using a spirit lamp[15].

Inoculums' preparation:

The inoculum was held to a standard of 0.5 McFarland turbidity.

Swabs preparation:

At 37 OC for 24 hours, a prepared and autoclaved cotton swab was used. during the entire sub-culturing of the wire process.

Media preparation and sterilization

The 5.04 grammes of nutritional agar were dissolved in 180 millilitres of distilled water (28 grammes per litre) in a flask. Cover the flask with a cotton plug once they have completely dissolved, and then allow it to autoclave at 121 °C for 20 minutes. The media was transferred to petri plates after autoclaving. Agar solidified after each petri plate received 20 ml of sterile solution, which was then allowed to cool for an hour at room temperature. The well diffusion method, which is described with little modification, was employed to test the antibacterial

activity of a chosen plant. The antibacterial activity of the crude extract of the supplied plant was tested using four different bacteria strains. A total of 12 petri plates were autoclaved for 20 minutes, and bacteria were then lawned on each plate by dipping cotton swabs into test tubes containing inoculums that had been sitting for 24 hours at an angle of 600C. These plates were then labelled. Four wells with a 6mm diameter were created in four plates using a cork borer. Using a micropipette, plant extract was added to wells along with a negative control (methanol) and a positive control—the standard antibiotic Vancomycin (VA30). The agar plates were incubated for 24 hours at 37 OC. The inhibitory zone created by each plant extract was measured with the aid of a ruler in millimetres (mm) after a 24-hour incubation period [16].

2.3 Antifungal Activity

In a flask that had been shaken, the 7.02 g of Potato Dextrose agar were dissolved in 100 ml of distilled water. The prepared media was then placed to a petri plate and autoclaved for 20 minutes. Before setting up the media, the plant extract was spread over the whole surface of the petri plates, covering them entirely. The fungal strain was carefully positioned in the centre of the plates after the media had solidified. The plates were then covered with a lid and kept at room temperature for 7 days. The outcomes were measured after seven days.

2.4 Antioxidant (DPPH Free Redical Scavenging) Activity

` Having a few exceptions, a free radical is any atom or molecule with a single unpaired electron in its outer shell and is highly reactive. Free radical damage and oxidative damage are tightly associated for the majority of biological structures. Antioxidants are reducing substances that prevent free radicals from damaging biological structures by passivating them against oxidative damage. The chemical compound DPPH (2, 2diphenyl-1-picrylhydrazyl) is made up of stable free radical molecules and appears as a dark-colored crystalline powder. In antioxidant assays, DPPH is frequently utilised. In the current investigation, the potential of the various plant extracts to act as DPPH radical scavengers were assessed [17, 18].

Principal

The free electron pairs up in the presence of a DPPH free radical scavenger, which decreases absorption and causes stoichiometric decolonization from deep violet to yellow.

Requirments

Spectrophotometer, incubator, micropipette, test tubes, DPPH radical, plant extracts, etc.

Procedure

A modified version of the DPPH method was used to test the antioxidant activity of the plant extract. Carefully produced 3.9 mg DPPH solution was used in 25 ml of methanol. Given that it is light-sensitive, foil should be used to protect it. Then 4ml of the DPPH solution and 1ml of the plant extract were combined and shaken. The combination was then left to incubate at room temperature for around 30 minutes in the dark. After the incubation period, the mixture's absorbance was measured using a spectrometer set to UV at 570 nm.

%DPPH was determined using formula:

$$\text{DPPH\%} = Ao - As/Ao \quad \text{x} \quad 100$$

As opposed to A0, which is the absorbance of the reaction mixture as well as 0.001 M of DPPH solution alone.

2.5. Cytotoxic Activity (Brine Shrimp Lethality Assay)

The COMSATS University Abbottabad Campus provided brine shrimp eggs. A plastic container with a hatching chamber (a divider for a dark (covered)/light region) was filled with artificial saltwater (FILTERED) that had been made by dissolving 38g of sea salt in 1000ml of distilled water. Brine shrimp (eggs) were placed on one side of the chamber (the dark side), and the hatchling shrimp were linked to the light-side of the chamber. The shrimp were given two days to hatch and grow into larvae. After 48 hours, 10 brine shrimps (30 shrimps per dilution) were added to each test tube containing the shrimp larva, and the artificial seawater volume was increased to 5mL by adding 4mL. Ending the lamp, the test tubes were not covered. Every 24 hours, the number of dead shrimp was counted and recorded. The lethal dose was determined at the previously stated 95%CI using probit analysis [19].

3. Results

3.1. Phytochemical analysis

Various solvents, including ethanol, methanol, and distilled water, were used. The findings indicated that alkaloids were present in the stem of the ethanol solvent, whereas alkaloids were present in the stem and leaves of the methanol and distilled water solvents, but not in the root extract. While other sections do not contain anthraquinones in all solvents, leaves contain anthraquinones in both ethanol and methanol. Cumarine are lacking in distilled water solvent but present in all regions of the ethanol solution and the leaves and stem of the methanol solution. All of the components in each solvent contain flavonoid compounds, but none of the components in each solvent contain glycoside, oil, or pholobutannins. Other plant compounds, such as phenols, quinones, steroids, saponins, and tannins All of Cirsium swaticum Petr's sections contain tannins and terpenoids in each solvent, as shown in (Table 1).

Table 1: Phytochemical analysis of *Cirsium swaticum*

S.No.	Chemical compounds	Methanol	Ethanol	Distilled water
1	Carbohydrates	+	+	+
2	Tannins	+	+	+
3	Flavonoids	_	_	_
4	Quinones	+	+	+
5	Glycosides	+	+	_
6	Cardiac glycosides	+	+	+
7	Terpenoids	+	+	+
8	Phenols	+	+	_
9	Coumarins	_	_	_
10	Phlobatanins	+	+	+
11	Anthraquinones	_	_	_
12	Oil	_	_	_

Key = Negative sign **(-)** indicate absence, positive sign **(+)** indicate presence

3.2. Anti-bacterial activity

The Cirsiumswaticum Petr leaves' methanolic extract significantly inhibited Escherichia coli bacteria, with a mean zone of inhibition of 24 mm and a Standard Error mean (SE) of 0.57 noted. The mean zone of inhibition against methicillin-resistant Staphylococcus aureus was measured at 25 mm, with a SE Mean value of 2.30. The mean zone of inhibition for Cirsiumswaticum Petr against Pseudomonas aeruginosa bacteria was 18.667 mm, and a SE Mean value of 2.33 was noted. The antibacterial activity against Salmonella typhi bacteria revealed that the average zone of inhibition was 30 mm, with a SE Mean value of 1.7321, as shown in (Table 1 & Fig. 1). Comparatively to the positive control treatment, which employed the standard antibiotic Vancomycin (VA30), the leaf extract exhibits the highest bacterial activity against all of the selected bacterial strains, as seen in (Table 2 & Fig. 2).

Table 2: Antibacterial activity of *Cirsium swaticum*

S.No	Bacterial Strains	Different Concentrations (ZOI mm)			Control
		1:10	1:20	1:40	
1	*Proteus mirabilis*	15	18	20	23
2	*Bacillus subtilis*	16	20	22	26
3	*Bacillus megaterium*	18	19	23	20
4	*Staphylococcus aureus*	20	21	25	26
5	*Salmonella typhi*	20	30	25	27
6	*E. coli*	12	15	17	27

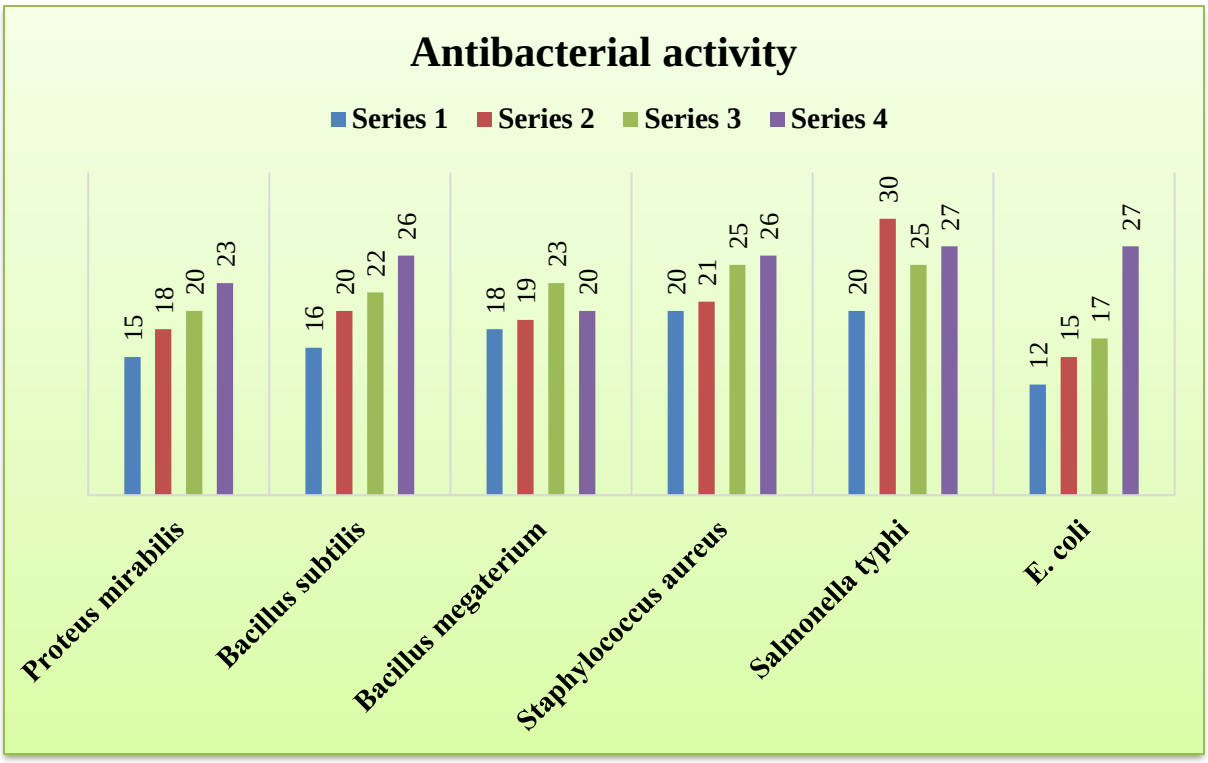

Fig 2: Antibacterial activity of *Cirsium swaticum*

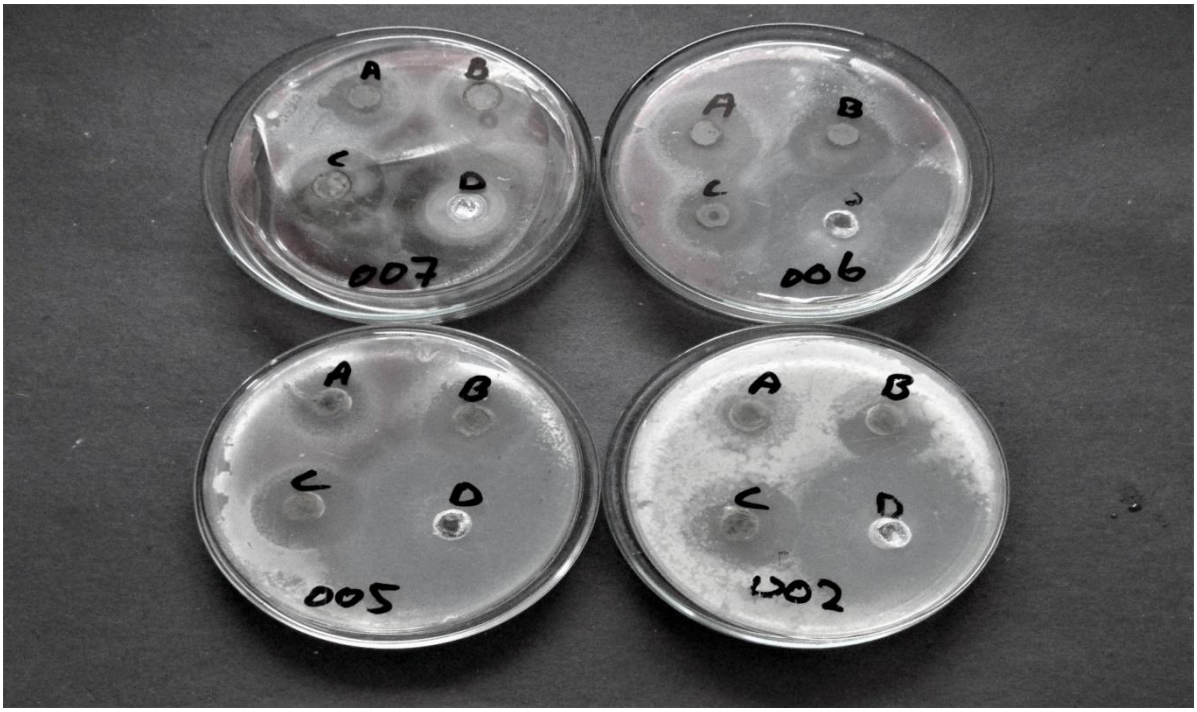

Fig 3: Antibacterial activity of *Cirsium swaticum*

3.3. Antifungal Activity

In contrast to other fungal strains, including *Alternaria Sp, Carvolaria Sp, Fusarium Sp, Helminthosporium Sp and Rhizopus Sp*, the crude methanolic extract of Cirsium swaticum Petr was also seen. The crude methanolic extract of the powdered leaves of the chosen plants displayed extreme Region of Reticence in divergence to *Alternaria Sp*. with 20 mm reticence zone as relate to Standard drug (Terbinafine) with 30 mm shyness zone, tailed by *Fusarium Sp*. (18 mm) as compare to regular medicine (20 mm), *Helminthosporium* (17 mm) as compare to Terbinafine (19 mm), *Rhizopus SP*. (17 (23 mm Zone of Inhibition). The figure displays the final outcome.

Table 3: Antifungal activity of *Cirsium swaticum*

S. No	Fungal Strains	Extract ZOI (mm)	Control
1	*Alternaria* Sp.	20	30
2	*Carvolaria* Sp.	16	23
3	*Fusarium* Sp.	18	20
4	*Helminthosporium* Sp.	17	19
5	*Rhizopus* Sp.	17	21

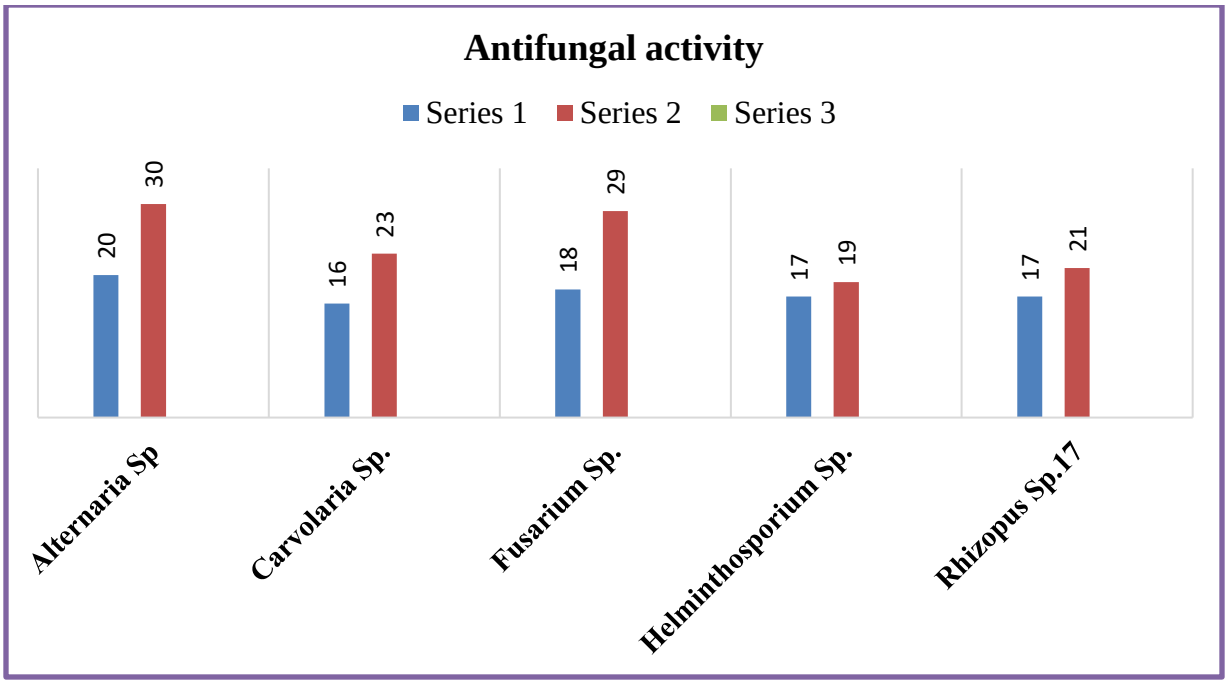

Fig: Antifungal Activity of *Cirsium swaticum*

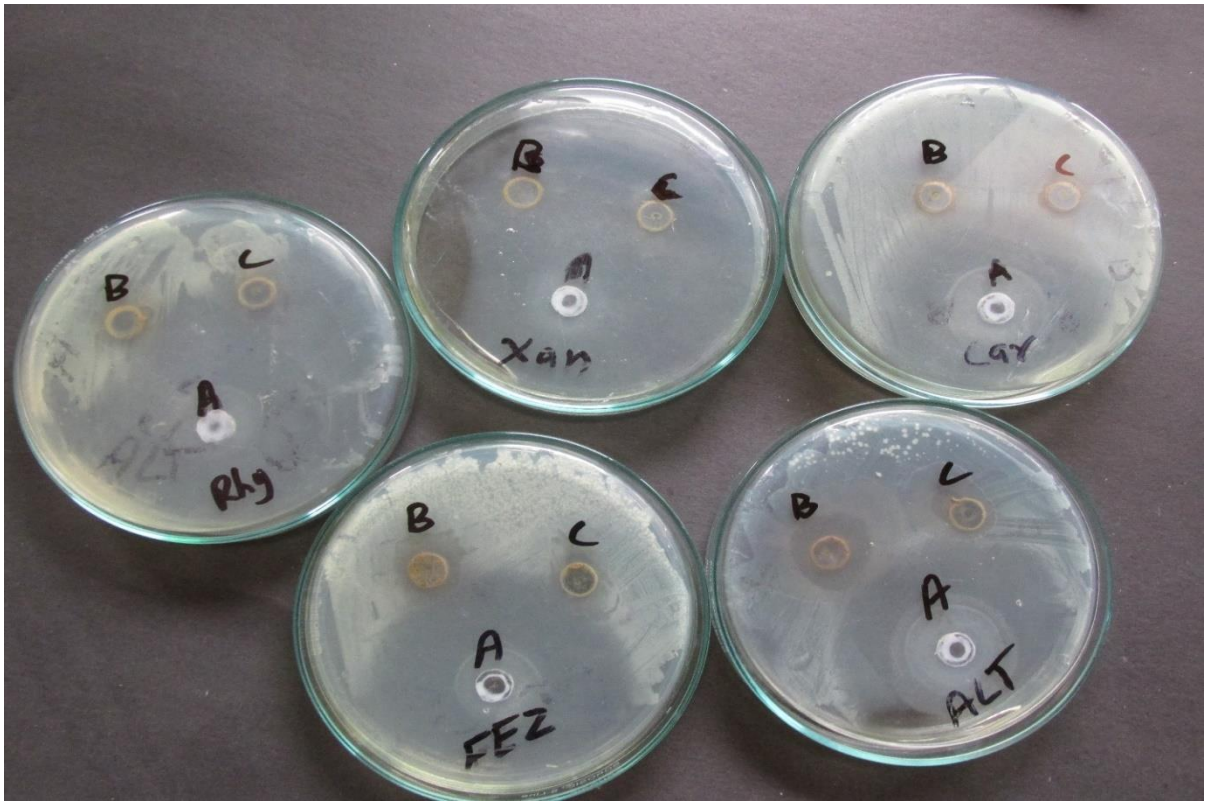

Fig 3: Antifungal Activity of *Cirsium swaticum*

3.4. Antioxidant Activity

The anti-oxidant Cirsium swaticum Petr's ability to scavenge free radicals was tested using standard DPPH, and the inhibition was assessed at several concentrations, including 1, 3, and 5 mg/ml. The results showed that the crude methanolic extract of Cirsium swaticum Petr at concentration 1 mg/ml displays the largest percent inhibition (70%), whilst the least per hundred inhibitions were registered at concentration 5 mg/ml (19%), for detail result see the (Table 4).

Plant part used			**Concentration (mg/ml)**	**Optical density**	**Inhibition%**
Ascorbic Acid			01	0.0857	91%
			03	0.0870	91%
			05	0.0533	94%
Cirsium swaticum	1	0.2965	70%		
	3	0.3914	60%		
	5	0.8040	19%		

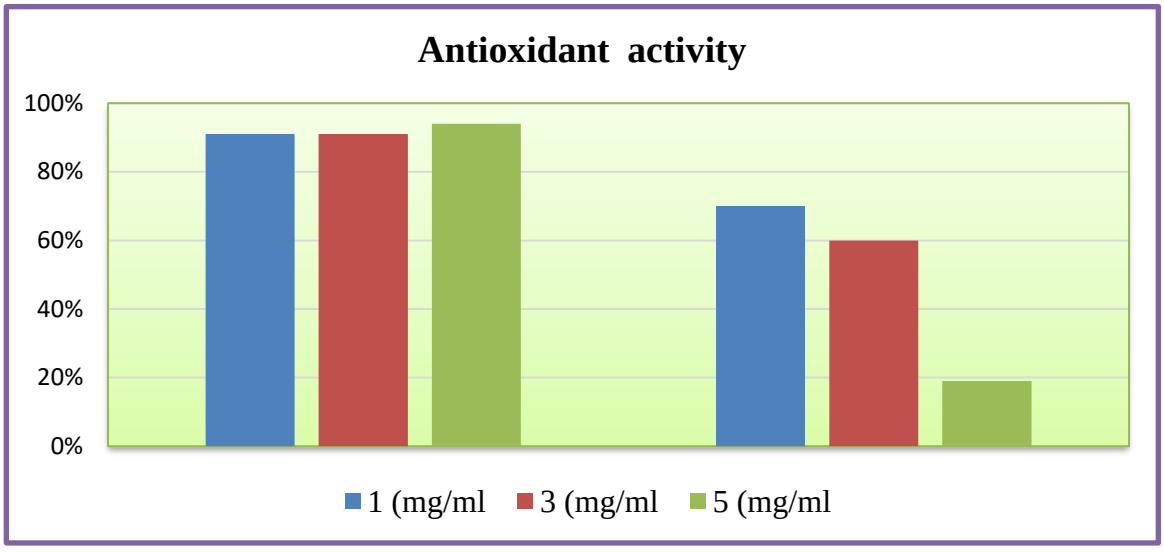

Fig 4: Antioxidant free radical scavenging activity *Cirsium swaticum*

4.5. Cytotoxic Activity

Test subjects for the current cytotoxicity investigation were brine shrimp larvae. The results showed that when the Cirsium swaticum Petr methanolic extract was tested at different concentrations of 100, 500, and 1000 ug/ml, respectively, the largest percentage of deaths was

recorded at 1000 ug/ml, which was 90%. The highest death rate was reported at 100 ugm/ml dose with just 30%, followed by 500 ugm/ml with 60% dying. Aside from the control, the maximum death rate was reported at 1000 ugm/ml dosages with 30%, while the lowest death rate was recorded at 100 ugm/ml doses with 10%. The outcome is displayed in table number.

Table 5: Cytotoxic Activity of *Cirsium swaticum*

Doses (µg/ml)	Plant part used	Total No. of shrimps	Shrimps lives	Shrimp died	Died percent
	Control		09	01	10%
100		10	07	03	30%
500		10	07	03	30%
1000		10			
	Whole plant				
100		10	07	03	30%
500		10	04	06	60%
1000		10	01	09	90%

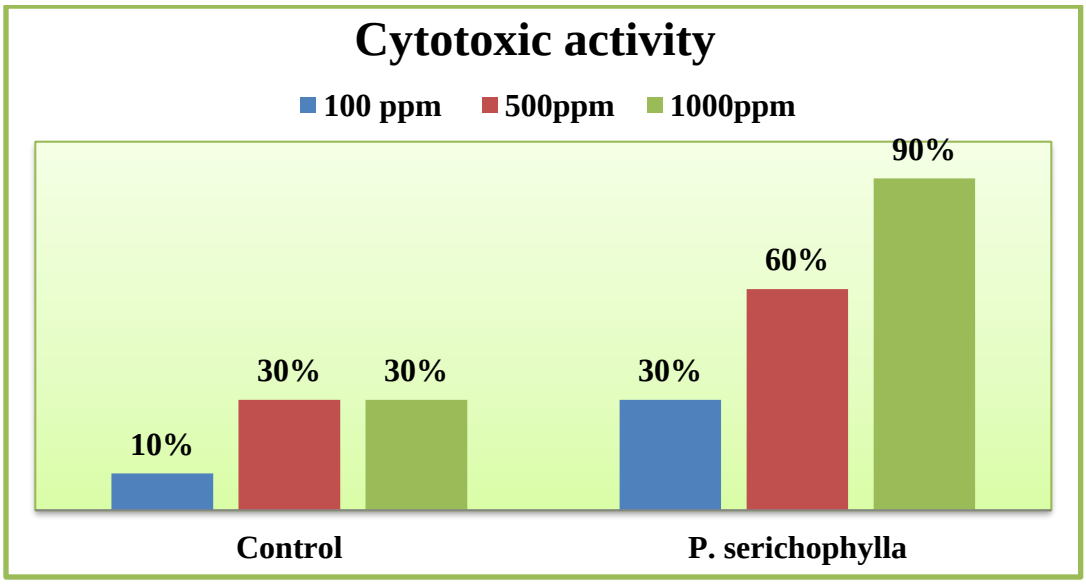

Fig 5: Cytotoxic Activity of *Cirsium swaticum*

Discussion

The goal of the previous lesson was to assess *Cirsium swaticum* Petr's antibacterial efficacy and phytochemical composition. The gramme positive and gramme negative bacterial strains Proteus mirabilis, Bacillus subtilis, Bacillus megaterium, Salmonella typhi, Staphylococcus aureus, and E. coli were compared to the crude methanolic extract of Cirsium swaticum[20] .The Staphylococcus aureus and Salmonella typhi (20 mm) were recognised as having the most extreme reserve during investigation, followed by Bacillus megaterium (18 mm), Bacillus subtilis (16 mm), and Proteus mirabilis (15 mm), while E. choli had the smallest reserve area (12 mm). At 20 l/ml, S. typi (30 mm) had the largest zone of inhibition, followed by S. aureus (21 mm), B. subtilis (20 mm), B. megaterium (19 mm), and Proteus mirabilis (18 mm), while E. coli had the smallest zone of inhibition (15 mm ZOI) [21]. Furthermore, Additionally, at a concentration of 40 l.ml, the all-out reticence precinct was observed in S. aureus (25 mm) and S. typhi (25 mm), followed by B. megaterium (23 mm), B. subtilis (22 mm), and Proteus mirabilis (20 mm), while the least hang-up precinct was observed in E. coli (17 mm ZOI) as opposed. The current study was contrasted with a report written by [22]. The antifungal potential of Cirsiumswaticum Petr was also examined in the current study in comparison to several fungal stains, such as Alternaria Sp., Fusarium Sp., Helminthosporium Sp., and Rhizopus Sp. The results showed that Alternaria Sp. (20 mm) had the most exciting region of reserve, followed by Fusarium Sp. (18 mm), Helminthosporium Sp. (17 mm), and Rhizopus Sp. (17 mm), while Carvolaria Sp. (16 mm ZOI) had the lowest zone of inhibition in comparison to Terbinafine (30, 23, 21, and 20 mm ZOI), respectively [23]. The present investigation was compared to reports presented by [24]. The antioxident permitted crucial Cirsium swaticum Petr foraging activity, which was examined by conventional DPPH. The inhibition was assessed at various concentrations, including 1, 3, and 5 mg/ml. The results showed that the crude methanolic extract of Cirsium swaticum Petr at concentration 1 mg/ml exhibits the largest percent inhibition (70%), while the least percent hang-up were logged at concentration 5 mg/ml (19%) [25]. For a detailed result table, please refer to the following [26]. The findings are significantly more similar to those from earlier investigations on the DPPH free radical scavenging activity of crude methanolic extracts [27].

Conclusion

Therefore, it is hoped that this plant will be taken into consideration in the future for additional clinical research, potential applications, and as an adjuvant to existing medications. These additional biological activities include those that are anti-inflammatory, analgesic, anti-spasmodic, and phytotoxic. Getting the active ingredients out of Cirsium swaticum Petr Decide which of the food's many nutritional components can be used in future studies.

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