In-Vitro Phytochemical analysis, Antimicrobial, Antioxidant and Cytotoxic activity of *Carpesium abrotanoides*

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Abstract: *Carpesium abrotanoide* L. Plant was examined for its biological activities, which included potential for antibacterial, cytotoxic, and antioxidant effects, as well as its phytochemical analyses. According to results of a phytochemical test, Several main and secondary metabolites are lacking in it. Carbohydrates, phenols, saponins, tannins, alkaloids, terpenoids, cardiac glycosides, and phlobatannins are all present in methanolic extract. Oil, glycoside, courmine, and glycoside—all of which were present—were absent from the mixture. It was determined that Escherichia coli, pseudomonas areuginosa, Klebsiella pneumoniae, Acinetobacter baumanni, and Staphylococcus aurus could not grow in the presence of the plant's methanolic extract. However, when tested in methanol against three fungi strains, including Mycospharellacitri, Penicillium, and Trichophyton mentagrophytes, the plant's extract displayed strong antifungal activity against each strain. The potential antibacterial properties of carpesium abrotanoide have been proven, and scientists anticipate that it may one day be employed to treat a variety of diseases and conditions. The experiment using brine shrimp deaths was used to gauge the plant's cytotoxicity (BSLA). It was demonstrated that the herb had cytotoxic effects. The antioxidant capacity of the plant was evaluated using the DPPH method. An extract of the herb Carpesium abrotanoide in methanol was found to have antioxidant properties. Future medical applications will make use of the plant's development results.

Keywords: Phytochemicals, Antimicrobial, Cytotoxic and Antioxidant activity of *Carpesium abrotanoide* L.

Introduction:

The role that medicinal plants play in the separation and development of novel medications is extraordinary. There growing is interest in employing medicinal plants as part of various traditional remedies to treat various illnesses [1, 2, 3]. The main drivers for the revival of herbal medicine are the negative side effects high cost and therapeutic limitations of currently available pharmaceuticals [4]. The use of plant extracts or their phytoconstituents is a key component of traditional medicines, which are used by more than 80% of people around the

world as their primary source of healthcare [4]. Plant-derived metabolites have recently gained significant importance as a result of their diverse structural makeup and intriguing bioactivities [5,6,7]. There are roughly 21 species in the Carpesium genus (Asteraceae) worldwide [8]. Both Europe and Asia are home to its species with the mountainous Southwest China being the most abundant [9]. In several Asian countries, its plants are used as a traditional treatment for a variety of ailments, including colds, fevers, insects, bruising, inflammation, and snakebites [9]. C is one of the most often studied species phytochemically. C. cernuum L. L., C. Abrotanoides miq., C. rosulatum C. Lipsky and C. Winkl. glum Maxim, C Franch's macrocephalum. & Sav., C. C. rosulatum and Miq. Sieb, divaricatum & Zucc. They are said to biosynthesize metabolites with a wide range of structural variations, such as mono-, sesqui-, and di-terpenoids, flavonoids, and phenolics [9]. These metabolites exhibited antioxidant, cytotoxic, insecticidal, anti-parasitic, anti-inflammation, anti-tumor, and anti-parasite activities [9]. The plant is a perennial herb with several branches and simple, elliptic leaves that are arranged alternately. It has tiny, branching flower clusters called capitula that emerge from the leaf axils [10]. Each capitulum has 130–300 golden, minute, closely packed florets that grow into longitudinal, cylindrical, ribbed achenes (fruit), which are about 1 mm in diameter and 3.5 mm in length [10]. C. Abrotanoides have a wide range of bioactivities, including cytotoxic, antiparasitic, insecticidal, antidiabetic, antioxidant, and anti-inflammatory effects. They have been employed in traditional medicine for treating a variety of health issues. Additionally, it was discovered to produce a variety of metabolites with interesting bioactivities, including sesquiterpenes, dimeric sesquiterpenes, monoterpenes, sterols, and aliphatic and nitrogenous chemicals. There are no existing review articles that deal explicitly with the role that abrotanoides play in traditional Asian medicine. Due to this, the present review compiled all available information on the phytoconstituents, traditional applications, bioactivities, toxicity, and documented biosynthesis and synthesis of the plant's metabolites. The researchers can learn about the most recent advancements in C research through this review. They can quickly identify secondary metabolites and assess their potential biological action with the use of abrotanoides. We also hope to raise awareness of one of the most significant medicinal plants and pique the attention of natural products researchers in order to further explore and employ the identified metabolites from it as therapeutic leads. This effort may also expose the current gaps and indicate the requirement for C. Conventional use of aborotanoides in scientific verification. Determining the possible therapeutic uses for C that have

not yet been investigated may also benefit from summarizing the reported bioactivities. Abrotanoid compounds or its components.

Fig 1: *Carpesium Abrotanoides* L.

2. Materials and Methods

The *Carpesium Abrotanoides* L. fresh leaves, bark and root were harvested in Pakistan's District Abbottabad. The collection area's coordinates are 34.5482° N and 73.3532° E. They were identified by Prof. Dr. Ghulam Mujtaba Shah, Chairman, 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.

2.1. Phytochemical analysis

As solvents, ethanol, methanol, and distilled water were used to test the crude extracts for the presence of various chemicals. The leaves, stem, and root were prepared in a solution of 1:10 ethanol, methanol, and distilled water. The five grammes of powdered drug was then steeped in 50 cc of the aforementioned solvents, mixed often each day, and allowed to soak for three to four days. The filtrate was then used for several phytochemical studies, including those on sapon,

flavonoids, quinine, tannins, carbohydrate, alkaloids, glycoside, terpenoids, phenols, cumarins, pholobutannins, authroquinones, and altered oil and lipids. Afterward, Wattman's filter paper was used to filter the filtrate [11, 12, 13, 14]

Tests

A chemical reaction involving water (H2O), iodine solutions (Mayers regents), sodium hydroxides (NaOH), ferric chlorides (FeCl3), potassium hydroxides (KOH), chlorine (CHCL3), and hydrochloric acids (HCL3) produces acetic acid (CH3COOH). all included in this grouping (HCL).

Chemical required

The Concentrate containing sulfuric acids (H2SO4), acetic acids (CH3COOH), iodine solutions, mayers regents, distillate water (H2O), ferric chlorides (FeCl3), potassium hydroxides (KOH), sodium hydroxides (NaOH), chloroform (CHCL3), and hydrochloric acid (HCL).

2.1.1 Carbohydrates Test

In an experiment tube, two millilitres of plant extracts and two millilitres of iodine solutions were added to ascertain whether the plant contained carbohydrates. Carbohydrates can be distinguished by their blue colour.

2.1.2 Test for Flavonoids

To detect the presence of flavonoids, two ml of concentrated 2 ml HCL were added to a test tube with two ml of plant extract (hydrochloric acid). Drop by drop, Mayer's reagents were also added. Flavonoids are responsible for the vivid green hue.

2.1.3 Test for Tannins

The presence of tannins was detected after adding ferric chloride (Fecl3) to 2ml of plant extract in a test tube.

2.1.4 Test for Saponins

Plant extract and distillate water were combined in a test tube and aggressively agitated until bubbles appeared. The combination was then allowed for 10–12 minutes.

2.1.5 Test for Quinone

To check for the presence of quinones in the plant, one ml of concentrated H2SO4 and one ml of ethanolic plant extract were put into a test tube. Quinones are distinguished by their deep scarlet colour.

2.1.6 Test for Glycoside

The 10% sodium hydroxide was combined with a small amount of plant extracts and the chloroform before being put into a new container. Glycosides can be found in the 10% sodium hydroxide, which is created by mixing 10ml sodium hydroxide with 90ml distilled water.

2.1.7 Test for indication of Cardiac Glycosides

The plant extract was placed in the test tube together with two ml of acetic acid, few drops of ferric chloride, and one ml of sulphuric acid. The borders of the tube display bands that represent cardiac glycosides.

2.1.8 Test for Terpenoids

The plant extract, which was diluted with two ml of chloroform, has a reddish colour, which indicates the presence of terpenoids.

2.1.9 Test for Phenols

One millilitre of the plant extract, two millilitres of deionized water, and a few drops of a 10% FeCl3 solution should be placed in a test tube to check for the presence of phenols (ferric chlorides). The appearance of blue or greenish things is a sign that phenol is present.

2.1.10 Test for Coumarins

One millilitre of plant ethanolic extract and one millilitre of 10 percent sodium hydroxide (NaOH) solution added drop by drop to a test tube can detect the presence of coumarins.

2.1.11 Test for Phlobatannins

The presence of a crimson hue in the ethanolic plant extract that was placed in a test tube with 2% HCL indicates the presence of phlobatannins.

2.1.12 Test for Anthraquinones

The appearance of a crimson hue in the ethanolic plant extract that was added to a test tube containing 2% HCL shows that phlobatannins are present.

2.1.13 Test for Oil

For the oil test, filter paper was employed. The filters' papers' damp look indicates that there may be some oil presents.

2.2 Antibacterial Activity

The agar well diffusion technique was used in accordance with accepted guidelines to ascertain its antibacterial capabilities [15, 16].

2.2.1 Basic Principle

This technique caused antibiotic components from plant extracts to spread from the media and correlate with a test bacterium. It is possible to see the development of inhibition around the wells and it is measured in millimetres. There are circular rings of inhibition.

2.2.2 Material required

This experiment required the use of sterile borers, dimethyl sulphoxide, sterile water, and a micropipette in addition to Petri plates with several bacterial strains.

2.2.3 Bacterial Strains

For its antibacterial qualities, different bacterial strains were chosen and gathered from the Department of Botany, Hazara University Mansehra Pakistan. In order to preserve the bacteria, they were created in nutritional broth and then cultured. Five gram-negative bacterial strains and one gram-positive bacterial strain was used (Table 1).

Table .1 Bacterial strains causing various diseases

S. No	Bacterial Strain	Type of bacterial strains	Diseases caused
1	*Staphylococcus aureus*	Gram +ive	Dermatitis , endocarditis
2	*Escherichia colii*	Gram –ive	Urinary tract infection, diarrhea, meningitis
3	*Pseudomonas aeruginosae*	Gram –ive	Respiratory,system,infections, gastrointestinals infection
4	*Klebsiella pneumonia*	Gram –ive	The wound or surgical site infections
5	*Salmonella typhii*	Gram –ive	Typhoid, food bornes sickness, paratyphoid
6	*Acinetobacter baumanniie*	Gram –ive	Blood, infections, urinary, tract infections lungs infections or pneumonia

2.2.4. Antibiotics

Ciprofloxacin was one of the medicines used in an antibacterial action against microorganisms.

Method

2.2.5 Preservations of a Bacterial and Fungal strains.

The microorganisms were placed in petri dishes with nutritional broth to promote bacterial and fungal growth, and they were then incubated for 24 hours at a temperature of around 37 0 C. The distinct microbial strains were grown in separate tubes.

2.2.6 Preparations of stocks

To make stocks solutions, 1g of plant extract was diluted in 1ml of dimethylsulphoxide, and dilutions were made by mixing 300 l of extract with 2.7 ml of distilled water.

2.2.7 Nutrients agar Preparations

1 litre of distilled water was used to dissolve 38 grammes of agar, which was then placed in an autoclave. The medium was then put into petri plates in a laminar flow after being autoclaved for approximately 15 minutes at 15 PSI pressures and 1210C temperatures. The Petri dishes were then incubated after that.

2.2.8 Agar well diffusion technique

Antibacterial analysis was carried out using agar well diffusion. 25–30 ml of media was placed in sterile petri dishes and left to solidify at room temperature. A sterile borer was used to create wells in a petri dish that were 6 mm in diameter. Using sterile cotton swabs, the different bacterial strains were dispersed. As positive and negative controls, a further 30 mL plant extract, 30 mL DMSO, and 30 mL of the antibiotic ciprofloxacin were added to the wells, and the plate was then incubated at 37 0C for 24 hours. In the distinct zones formed around the wells, bacterial growth was inhibited to varying degrees. After that, the zones formed around the wells were measured using the applicable techniques. It underwent three tests to determine whether it was antibacterial [15].

2.3 Antifungal assay

We carried out an antifungal assay using a standard method [16].

2.3.1 Antibiotic

Terbinafine was used to treat fungal infections.

2.3.2 Fungal strains

Some of the fungal strains used were *Aspergillus and other fungus, Penicillium, Rhizopus stolonifera, and Mycosphaerellacitri.*

2.3.3 Materials requireds for antifungal activity

A test tube, an incubator, an autoclave, plant extracts for SDA (Sabouraud, Dextrose, and Agar), antifungal drugs for fungi, and micropipettes.

2.3.4 Preparations of Nutrients media,

Sabouraud Dextrose Agar medium was created by combining 14 grammes of Dextrose with 60 litres of distilled water and injecting the mixture into test tubes for antifungal activity. The tubes were then sterilised for two minutes at a temperature of 1210C in an autoclave.

2.3.5 Stock solution Preparation

The DMSO solution and carpesium extracts were blended for a minute at a time on the gyro mixer. A stock solution and 300 ml of 2.7 ml of distilled water were used to further dilute the extract.

2.3.6 Methods

The method is same as antibacterial, assay (Agar well diffusions method).

2.4 Cytotoxic Activity

The Standard protocols were used to conduct the cytotoxic activity [17].

2.4.1 Required media

Brine, shrimps egg sea salts, distilled water a partitioned tray or containers plant extracts test tubes, micropipette, and a magnifying lens.

2.4.2 Preparation of stock solution

It was created by the dissolving 20mg plant extracts in the 2ml of ethanol.

2.4.3 Method

The plant's potential for cytotoxicity was evaluated using the Meyer, et al. Brine shrimp eggs were first placed in a plastic tray or container with a perforated divider after sea salt had been dissolved in 1000ml of water. To facilitate the hatching of the eggs, the container was maintained at a temperature of 34–36°C for a day. The shrimp migrated to the other side of the container after hatching, where they started swimming. The test tubes were filled with stock solution in three concentrations—10 ppm, 100 ppm, and 1000 ppm—and left for 24 hours to allow the ethanol in the test tubes to evaporate. Ten freshly hatched brine shrimp were then placed in each test tube, along with a micro-pipette and 2 millilitres of sea salt to create a total of 5 millilitres, and left for 24 hours. A magnifying glass was used to count the number of dead and alive brine shrimp the following day in each test tube.

2.5 Antioxidant Activity

Using the radical scavenger DPPH, it was demonstrated that ascorbic acid has substantially lower antioxidant activity. The antioxidant properties underwent standard methodological testing [18].

2.5.1 Material

The plant extracts were examined using a spectrophotometer, a DPPH detector, trichloroacetic acid (TCA), potassium ferric cyanide, and phosphate buffer**.**

2.5.2 Method

The DPPH technique's results were interpreted using the IC50 value. This graph shows the relationship between concentration and discoloration (IC50). A graph was utilised to quantify the percentage of inhibition extract concentration for each sample's antioxidant activity tested in IC50. This sample size is required for a 50% reduction in DPPH absorbance. Before the DPPH test was finished, some modifications to the standard protocol were performed. 1.5ml of 0.1mm DPPH solution was combined with plant extract concentrations (10–500g/ml). The mixture was then mixed and incubated in the dark for approximately 30 minutes. The ascorbic acid was utilised as the positive control and the absorbance at 517 nm was used to measure the amount of DPPH free radical reduction. After doing the operation three times, the average results were calculated. Using this calculation, we were able to calculate the amount of the free radical DPPH that the sample was inhibiting in comparison to the control sample. The test absorbance for the reaction mixture and the control absorbance are displayed [19].

Results

Qualitative phytochemicals screening of *Carpesium abrotanoides*

C. abrotanoides L. was examined utilizing the phytochemical screening approach for the presence of several organic compounds. All three extracts contained phlobatannins, quinones, cardiac glycosides, terpenoids, tannins, and carbohydrates (ethanol, methanol, and distilled water). Methanol and ethanol both contain phenols and glycosides, while distilled water doesn't. Carpesium extracts lacked any flavonoids, coumarins, anthracites, or oil (Table 2).

Table .2 Phytochemicals screening of powdered *Carpesium abrotanoides*

S. No.	Chemical compounds	Methanol	Ethanol	Distilled water
1	Carbohydrates	+	+	+
2	Tannins	+	+	+
3	Flavonoid	_	_	_
4	Quinone	+	+	+
5	Glycoside	+	+	_
6	Cardiac glycoside	+	+	+
7	Terpenoid	+	+	+
8	Phenol	+	+	_
9	Coumarin	_	_	_
10	Phlobatannin	+	+	+
11	Anthraquinone	_	_	_
12	Oil	_	_	_
13	Saponins	+	+	+

Present = + Absent = -

Antibacterial activity of *Carpesium abrotanoides*

The antibacterial activity of the plant's carpesium extract dissolved in methanol demonstrated the largest inhibition zone of 15 mm and the smallest inhibition zone of 12 mm when tested against Klebsiella pneumoniae and Staphylococcus aureus. The lowest inhibition zone for Escherichia coli and Staphylococcus aureus is 13 millimetres, compared to a maximum inhibition zone of 33 millimetres for Pseudomonas aeruginosa. The results are displayed in

Table 4.13 together with the antibiotic's and Carpesium extract's standard deviations. Since it has no effect on bacterial growth or inhibitions, DMSO is used as a -ive control Fig 23)

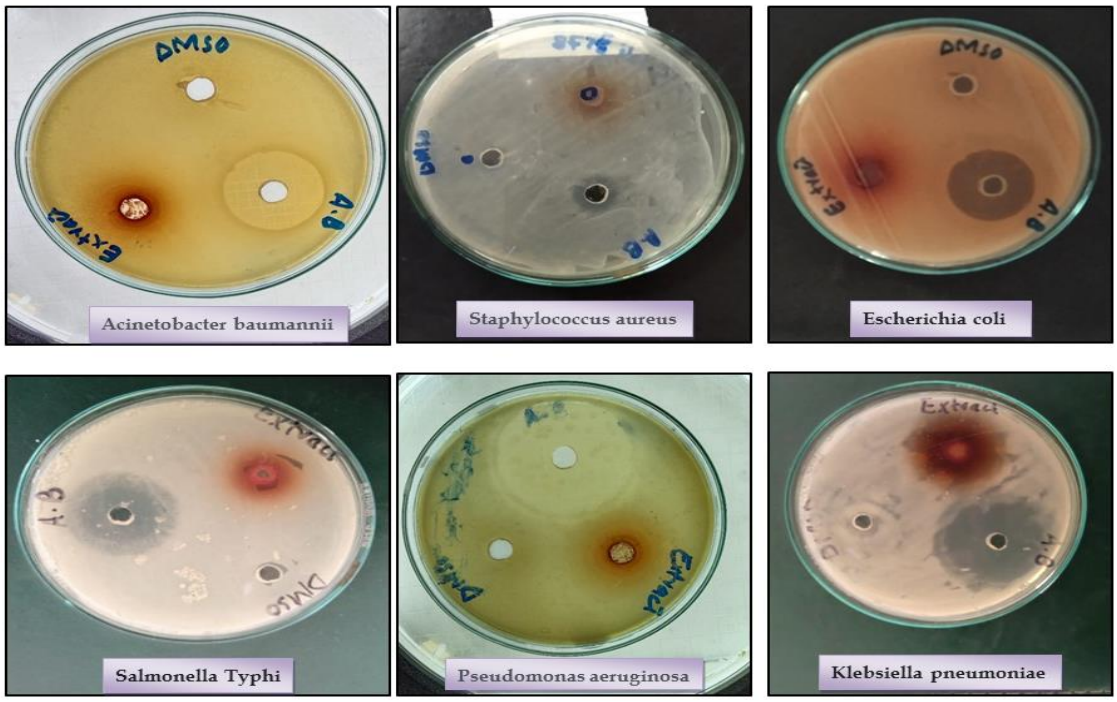

Fig. 2 Antibacterial activity of *Carpesium abrotanoides*

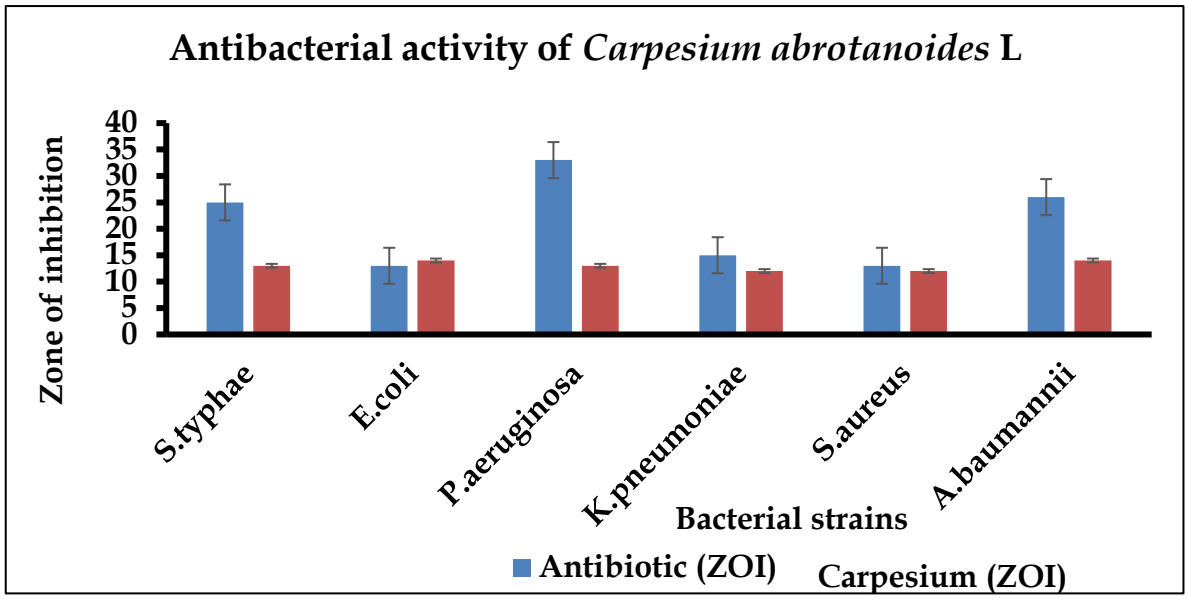

Fig. 3 Graphical representation of Carpesium abrotanoides

Antifungal activity of *Carpesium abrotanoides*

The Maximum inhibition zones against Rhizopuse stolonifera for the plant carpesium extracts were 18 millimetres, and minimum inhibition zones against Penicillium were 14 millimetres. In contrast to Penicillium, which had a maximum inhibition zone of 37mm, Rhizopus stolonifera displayed a minimum inhibition zone of 20mm.

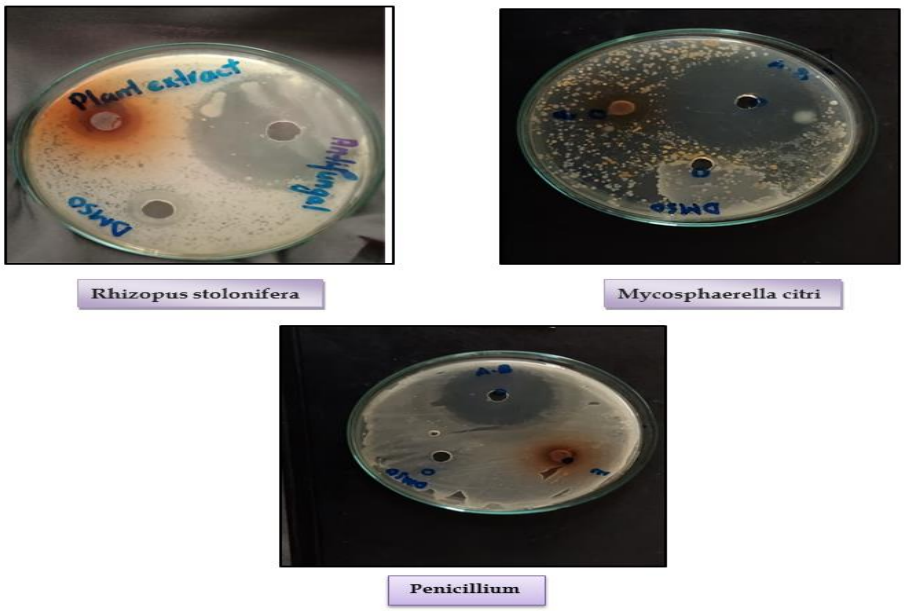

Fig. 4 Antifungal activity of *Carpesium abrotanoides*

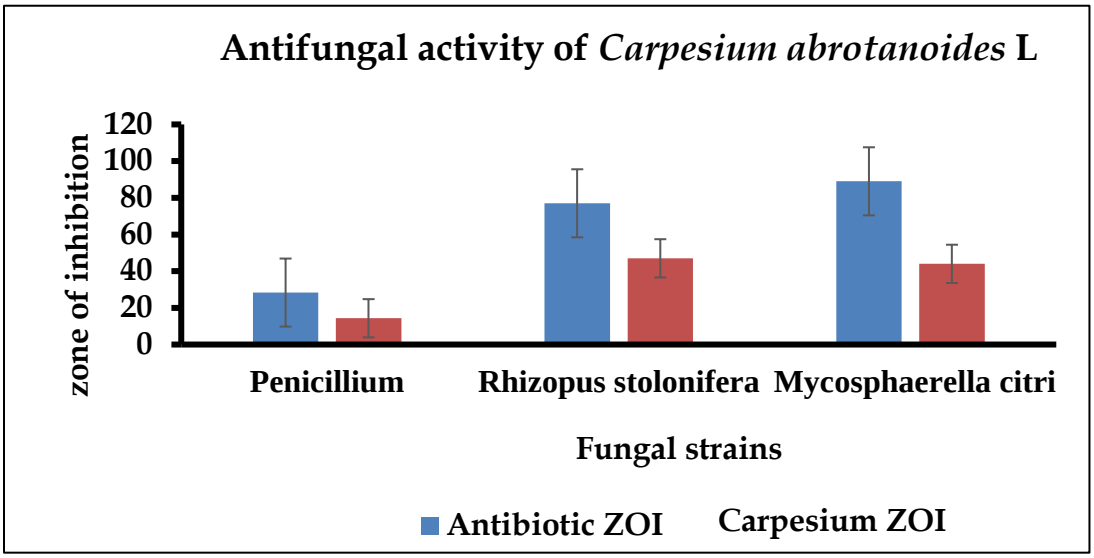

Fig. 5 Graphical representation of antifungal activity of *Carpesium abrotanoides*

Cytotoxic Activity

The methanolic Carpesium abrotanoides L. extract was applied to a larva's brine shrimp. At 10 ppm, just one shrimp was alive, and nine others were dead. At 100 ppm, the same was true, and at 1000 ppm, every shrimp had been exterminated. The maximum level of fatality was 100% at 1000 ppm. The plant's active components, which define its capacity for cytotoxicity, make it cytotoxic (Table 3).

Table .3 Cytotoxic activity of *Carpesium abrotanoides*

Concentrations	Plant Parts used	Replicates	Total no of Shrimp	Shrimps Lives	Shrimps Died	Dead%
10 ppm	Leaves,stem,roots	R 1	10	1	9	90%
100 ppm	Leaves,stem,roots	R 1	10	1	9	90%
1000 ppm	Leaves,stem,roots	R 1	10	0	10	100%

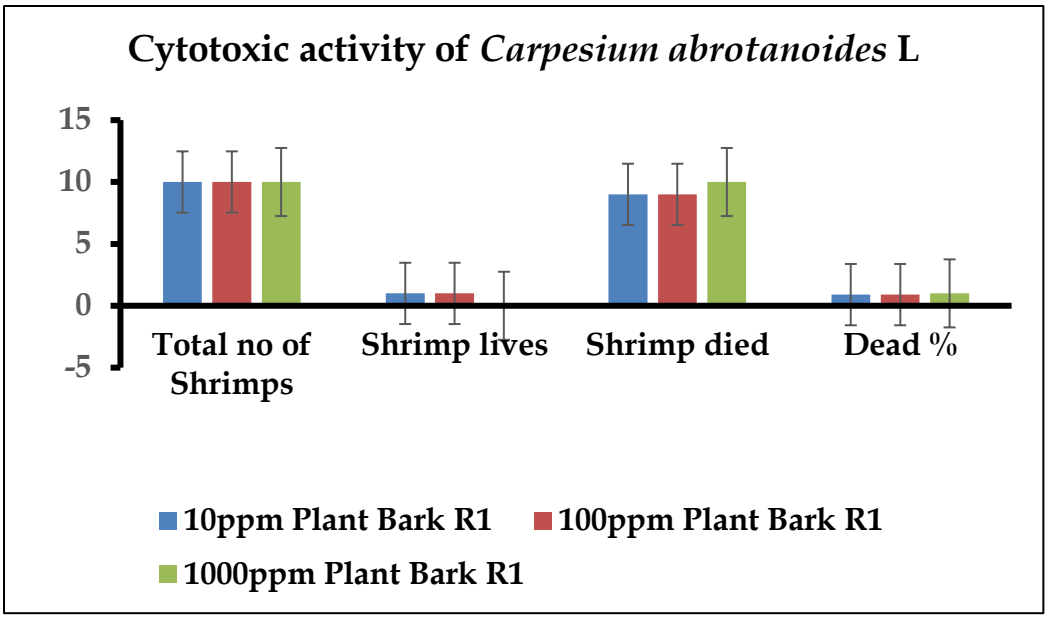

Fig.6 Graphicals representations of a cytotoxic activity of *Carpesium abrotanoides*

Antioxidant Activity

Carpesium abrotanoides was examined using DPPH at different concentrations, including 1000, 500, 250, and 125g/ml, and the inhibitions were analysed. A free radical scavenger, DPPH.

It appears that carpesium plant extract has the capacity to scavenge free radicals based on the decolonization of DPPH.

Antioxidant activity of *Carpesium abrotanoides*

Plant parts used (Methanol)	**Concentrations (µg/ml)**	**Mean ± S.E.M**	**IC $_{50}$**
Carpesium	1000	74.40 ± 0.38**	205
	500	60.80 ± 0.18**	
	250	48.50 ± 0.28**	
	125	49.94 ± 0.52**	

P=0.5**

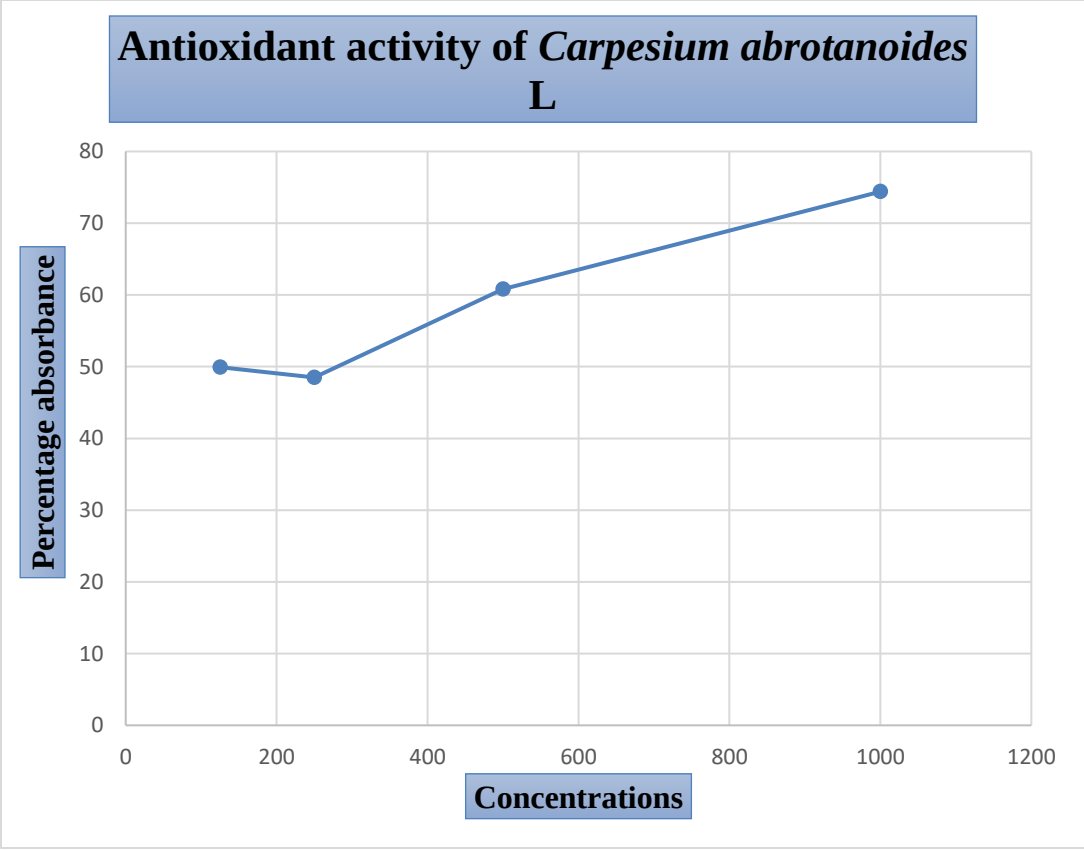

Fig .7 Graphical representation of antioxidant activity of *Carpesium abrotanoides*

Discussion

The plant extract has been found to have an abundance of phytochemicals, including several substances high in carbohydrates like tannins and quinones as well as cardiac glycosides such terpenoids and phlobatanins. Contrarily, fixed oil, anthraquinones, flavonoids, and coumarins were absent from carpesium. Plants have secondary metabolites that are biologically active. Plant secondary metabolites include terpenoids, flavonoids, terpenoids, and polyphenols, for instance. The healthcare system is significantly impacted by phytochemicals, which may also provide extra therapeutic benefits. Due to the anti-allergic, antibacterial, antispasmodic, antifungal, and antioxidant activity of these phytochemicals, the plant of this species has been utilised for alterative, astringent, depurative, discutient, emetic, expectorant, laxative, and vulnerable qualities [20]. In a study involving Escherichia coli and Carpesiumabrotanoides L, pathogenic bacteria such as Pseudomonas aeruginosa, Salmonellatyphi, Staphylococcus aureus, Klebsiella pneumoniae, and Acinetobacter baumannii had their growth hindered [21]. The results for bacterial strains are shown in the (Fig 3), and those for fungal strains are shown in Tables 4. The results are measured in inhibition in millimetres (mm). For example, gramme negative and positive bacteria such Pseudomonas aeruginosa, Escherichia coli, St. Acinetobacterbaumannii, Salmonellatyphi, and Klebsiapneumonia have all been demonstrated to be sensitive to methanolic preparations of carpesium [22, 23]. The methanolic extract of the plant produced a 15mm zone of inhibition and a minimum zone of inhibition of 12mm against Klebsiella pneumoniae [24]. Gram positive and gramme negative bacteria can both be treated with the plant's carpesium, proving that it has therapeutic benefits. In this investigation, it was discovered that the research plant Carpesiumabrotanoides L. has antibacterial characteristics [25]. It can therefore be used to treat a variety of bacterial infections. The methanolic extract of carpesium showed antifungal efficacy against Rhizopus, Mycosphaerellacitri, and Penicillium. Penicillium has a maximum zone of inhibition of 14 mm, compared to Rhizopus stolonifera maximum zone of inhibition of 18 mm [26, 27]. Carpesium abrotanoides The study revealed a relationship between pathogenic infections and infections. In the cytotoxic test, concentrations of 10 ppm, 100 ppm, and 1000 ppm were utilised, and the results revealed that 90% of the carpesium extract at 10 ppm, 90% at 100 ppm, and 100% at 1000 ppm were cytotoxic. So, at 1000 ppm, or 100%, the result was favorable. The data show that at 1000ppm, carpesium has the maximum cytotoxic potential and fatality rate. The antioxidant activity of Carpesium abrotanoides L. was

investigated using a traditional DPPH assay, and the inhibition was assessed at various dosages, ranging from 125 to 1000 mg/ml. According to the findings, carpesium extract has a sizable antioxidant activity [28].

Conclusions

Carpesium abrotanus L. possesses an exceptional qualitative phytochemical study of Carpesium abrotanoides L. showed the presence of active secondary metabolites like glycosides, polysaccharides, flavonoids, terpenoids, and alkaloids. There is proof that carpesium methanol extracts could be utilised as a source for testing new antimicrobial drugs to combat bacterial infections. The plant's cytotoxic activity is at its peak, according to recent research, at 1000 ppm. Antioxidant qualities of Carpesium abrotanoides L. have been displayed.

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