In-vitro Phytochemical Screening and Biological activities of bark of *Quercus glauca*

Meryam Iqbal *, Ghulam Mujtaba Shah*, Muhammad Fiaz*, Khursheed Ur Rahman *, Samia Abbasi*, Zain Ul Abdin *, Iqra*, Zabeeh Ullah*,

****Department of Botany, Hazara, University, Mansehra 21300, Pakistan***

Abstract: The Quercus glauca bark was examined for its phytochemical composition and biological activity, including its potential for antibacterial, cytotoxic, phytotoxic, and antioxidant effects. The methanolic extract of the bark's primary and secondary metabolites, including carbohydrates, phenol, tannins, saponins, terpenoids, alkaloids, phlobatanins, and cardiac glycosides, were identified using phytochemical analysis. However, there was no oil, courmine, glycoside, or glycoside. The bark's methanolic extract significantly inhibited the growth of five different bacterial strains, including Escherichia coli, pseudomonas areuginosa, Klebsiella penumoniae, Acinetobacter baumanni, and Staphylococcus aureus. The bark of the plant had notable antifungal results against all three fungal strains when its methanolic extract was tested against Mycospharella citri, Penicillium, and Trichophyton mentagrophytes. According to antimicrobial tests, the bark of Quercus glauca has the potential to treat a wide range of illnesses and disorders in the future. The Brine shrimp fatality experiment was used to determine the cytotoxicity of bark (BSLA). The plant's bark demonstrated cytotoxic properties. The bark of the plant was tested for phytotoxic activities against lemna minor L. plant may one day be employed as a weedicide. Through a DPPH free radical scavenging assay, the bark's antioxidant capacity was evaluated. Bark from the Quercus glauca tree demonstrated antioxidant potential when extracted with methanol. The plant has a lot of promise and may eventually be utilised for additional beneficial applications. This research can help identify and authenticate plants as well as species that could be employed in the future to create novel treatments.

Keywords: Phytochemicals, Antimicrobial, Cytotoxic, Phytotoxic and Antioxidant activity of the bark of *Quercus glauca* Thunb.

1. INTRODUCTION

Many plants create natural compounds that are manufactured to carry out a variety of biological functions for the plant, which they view as a gift of life. 30% of produced medicines are based on plants, and about 80% of people in the world depend on natural resources to preserve their health [1]. Herbal treatments are excellent sources of physiologically active

substances, particularly those with antioxidant and antibacterial properties [2]. Al-Reham, Al-Bakara, Al-Anam, Al-Momeenoon, Al-Aarafa, An-Nahal, and other surahs of the Blessed Book (Quran e Pakistan) have verses that go into length about the significance of plants. Herbal remedies were used and recommended by our beloved prophet Hazarat Muhammad (peace be upon him) for a range of illnesses and cuisines. The Hadith also makes reference to numerous plant species. About 70 medical plants and plant-derived products were cited by Farooqi (1998) in his book "Hadith Mein Mazkoor Nabatat, Adwiyah Aur Ghizaen," also known as "Plants, Medicines, and Food Mentioned in Hadith." [3]. The information on medicinal plants used by the inhabitants of the Eastern Cape to treat animal ailments was gathered using rapid rural assessment procedures, such as rating, storytelling, transect walks, seasonal calendars, and interviews [4]. Plants have long been thought to provide therapeutic benefits. Due to their wide range of applications, researchers have recently become interested in chemicals originating from plants [5]. Herbal remedies, which have a large proportion of biologically active chemical components that function as antibacterial agents, have been used for centuries to treat a variety of infectious disorders in humans. For outdated medical treatments, current therapeutic systems, pharmaceuticals, and synthetic chemicals, medical plants continue to be the most important bio-resources [6]. Since the beginning of time, man has used herbs or plant-based products as medicine to build immunity or resistance against colds, joint pain, fever, and other illnesses. Traditional medical practises like Ayurveda, Siddha, and Unani are built on the ability to treat common diseases using plant-based solutions. The vast majority of our population, particularly those in rural areas, continues to rely on herbal remedies. Numerous herbal remedies have stood the test of time, particularly when used to address aging-related metabolic, degenerative, and allergic diseases. Ayurveda and Unani medicine in the treatise did not, however, provide any scientific evidence on the identification or efficacy of these herbs [7]. The Quercus bark has significant therapeutic potential and is frequently employed in medicine as an antiseptic and an energizer. It is also advised for those who have bruises or haemorrhages, and it is administered as an injectable to those who have leucorrhea. In many parts of the world, it has also been utilised as a potent substitute for quinine in the treatment of chronic fevers. Additionally, the oak tree's bark helps treat chronic diarrhoea and dysentery, and a bark decoction can soothe a sore throat [8].

Fig 1: ***Quercus glauca*** **Thunb.**

2. MATERIALS AND METHODS

This plant is belongs to the family Fagaceae. In September 2020 it was collected from Kaghan valley, district Mansehra. The plant was recognized by Professor. Dr. Ghulam Mujtaba Shah, Chairman, Department of Botany Hazara University Mansehra, Pakistan. After identification the specimen were submittes in Hazara University (HUP) herbarium for permanent record. The plant materials were rinsed with tap water separated and make dry in shade for 15 days. These materials were used afterward for phyto-chemical and biological activities.

2.1 Phytochemical Examibation

50-60 gram of bark powder was drenched in ethyl alcohol for the phytochemical examination by following the protocol of with little modifications in it [9].

Qualitative phytochemical screening

2.1 Carbohydrate Test

In a test tube, two ml of bark extract was taken to examine the presence of carbohydrates in the back of plant. In a similar test tube, about 2ml solution of iodine was added. Blue or purple color appearance shows the existence of carbohydrates [10].

2.2 Flavonoid Test

In the test tube, 2ml of extract was taken with 2 ml of hydrochloric acid to check the existence of flavonoids Mayer's reagents were added to drop carefully. The dark green or white color indicates the presence of flavonoids [11].

2.3 Tannins Test

In 5% ferric dichloride's, 2ml of plant extract was added. A hazy green or shiny blue color indicates the tannins existence [12].

2.4 Saponins Test

Using one test tube, 2ml of bark extract and same proportion of sterile water was taken. For about ten minutes, shaken the test tube until the foams are formed 11].

2.5 Quinones Test

In the test tube, one ml of bark extract with same amount of concentrated H2sO4. The gloomy red color indicates the existence of Quinone [11].

2.6 Glycosides Test

About three ml chloroform and two ml of bark extract was added with 10% sodium hydroxide (NaOH), i.e. 90 ml of purified water for 10% of NaOH. The presence of glycosides known by the suggestion of pink color [12].

2.7 Cardiac glycoside Test

1 ml of bark extract was taken and, afterward, 2 ml of acidic corrosive was added with a couple of small globule of liquid of 05% ferric chloride and 01 ml of sulphuric acid. The groups shaping example at the very first look toward the finish of the cylinder shows the cardiac glycosides presence [11].

2.8 Terpenoid Test

After the enlargement of sulphuric acid, take two ml of chloroform with one ml of bark extract. Appearance of red color indicates the terpenoid presence [11].

2.9 Phenol Test

In a test tube, add 02 ml of distal water with a few drops of 10% phenol and two ml of extract to know the occurance of phenol in the plant. The greenish or blue color indicates the occurrence of phenol.

2.10 Phlobatannins Test

In a test tube, After 2 percent hydrochloric acid (HCL), one ml of ethanolic plant extract was added. The appearance of a red tint shows the phlobatanins presence [11].

2.11 Anthraquinone Test

In a test tube, one ml of bark extract was mixed with 10% basic arrangement. The appearance of a pink tint shows the anthraquinones presence [11].

2.12 Oil examination

By using the filter paper, the oil test was checked. By rubbing the powder on the filter paper, the sticky appearance shows the verification of oil [13].

Biological Assays

Preparation of Extract

The pieces of the plant were made powder. 50g of powder was drenched in methyl alcohol and then filtered, methyl alcohol is evaporated via rotatory evaporates at 40°C, and at room temperature it was kept for further use. The mention below biological activities were done.

Screening of antibacterial activity

The agar well diffusion technique was used by following the process with little alteration to check the antibacterial effect of bark of the research plant.

2.2 Crude Extract preparation

In 500 mL of methyl alcohol the bark powder in the proportion of 1:5 was soaked for one day. The filtrates were reserved at room temperature after filtration. The filtrates were then mixed and concentrated using a rotary apparatus at reduced pressure. The resultant particle was then pressed to dryness in a hot air drier to provide a powdered crude methyl alcohol extract [14].

2.2.1 Tested Bacterial Strains

Some of strains of bacteria were used to perform this activity included gram-negative (*Escherichia coli*, *Klebsiella penumoniae*, *Acinetobacter baumanni* and *pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*).

2.2.2 Procedure

At 120°C the medium was sterilized after the process of autoclaved was done. Each purified Petri plate is filled with 2ml of agar media which were kept for solidification at room temperature. By using the cotton swab the bacterial strains were apply over the agar plates. 6 mm diameter of sterile cork borer was used to make equal wells. 3 mg fraction of each extracts were dissolved in 1 mL of DMSO solution (20%). In a well 20µL of normal drug and plant fractions were poured. At 37°C, the petri dished were kept for incubation for about a day. For positive control the Ciprofloxacin was used while DMSO was used as a negative control. The inhibition zones where noted in millimeters.

2.3 Antifungal Activity

This experiment was done by following the procedure [15].

2.3.1 Nutrient media preparation

14g sabouraud dextrose agar (SDA) was liquified in 1000ml of distilled water and shaken, then is was added in test cylinders and kept in autoclave for sterilization. A stock solution was made. Plant extract was mixed in 1ml of DMSO. Distilled water was added for further dilution to the crude plant extract.

2.3.2 Method

By the agar well diffusion technique the isolated chemical was gauged for its antifungal properties against different strains of fungus. In decontaminated petri dishes the 50ml agar was poured and left for solidification. On these agar plates the sterile agar for about 100µL was spread. Used 6mm sterilized borer for making wells and saved in suitable spaces. After placing 100µL of the sample, the petri plates were left for incubation at 28°C for about 4 days. The inhibition zones of this activity were measured in millimeter (mm).

2.3.3 Fungal strains

There were three fungal strains were used as shown in (Table 1).

Table 1. fungal strains causing different diseases and infections

S.No	Fungal strains	Diseases caused by Fungal strains
01	*Penicillium*	*Penicillium* use to produce antibiotic called penicillin Caused skin infections
02	*Mycosphaerella citri*	Greasy spot disease in plants
03	*Trichophyton mentagrophytes*	Cause superficial skin infections

2.4 Cytotoxic activity

Brine Shrimp Lethality Test (BSLA)

This test is used to detect biologically active compounds in natural foods.

2.4.1 Reagent preparation

Take and label decontaminated serial dilution test cylinder. Weighed 10 mg of plant extract. The stock solution was prepared by adding 10 ml of bark extract in 1ml water. The stock solution was successively diluted in 1g/ml, 10g/ml, 100g/ml and 1000g/ml. From 1 to 5, three

tubes have been labeled. In 1 mL of prepared solution, 10ml of sea water and 10 nauplii were added. The number of lifeless nauplii had been counted after a day [16].

2.4.2 Procedure

Hatching brine shrimp

By using a measuring cylinder pour 3ml of water into a rectangular jar. Into the water-filled container, 28g of table salt was poured. Stir the water by using a spatula. To ensure appropriate aeration, into the bottom of the jar insert the tip of an airline air pump. Mix roughly about 20gram eggs of brine shrimp with the water at the jar's above surface of water. (60-100 watts) light bulb is used a few inches away from the jar. After 20 hours or a day the nauplii hatch. Watch over the eggs and nauplii. Collect the nauplii after a day. It's as simple as turning off the air and turning off the lights. In the water column the brine shrimp concentrated, whereas the empty egg floated. Transfer 10 nauplii to a test cylinder by using a Pasteur pipette.

2.4.3 Testing for toxicity

To different plant extract concentrations, the nauplii were exposed. Total the number of nauplii who lived and calculate the expiry rate after a day.

2.5 Phytotoxic activity

2.5.1 Analysis of phytotoxicity of Research plant

Lemna minor L. plant was used to check the phytotoxicity of this plant and protocol was used [17].

2.5.2 *Lemna minor* L. plant description

Lemna minor L. is the member of family Araceae and is commonly known as duckweed the smallest angiosperm. *Lemna minor* is found in the aquatic atmosphere, moving over the water, having three leaves and dangling roots. It shows rapid growth so it was carefully chosen for phytotoxic activity in contradiction of research plant.

2.5.3 Procedure

For phytotoxic examination, various salts were used for the preparation of media in distilled water. Preparation Stock solution was also done. Petri plates were used after the process of autoclave was done. Various concentrations of stock solution, media and 10 specimens of *Lemna minor* were transferred into petri plates for about a week. The number of fronds was calculated as compared to control after the completion of a week[17].

2.5.4 Formula

Percentage of inhibition = 100- (No. of fronds in extract of plant /No. of fronds in –ve control) ×100.

2.6 Anti-oxidant potential

DPPH free radical scavenging analysis

2.6.1 Procedure

Fading of a purple colored methyl alcohol solution assessed the hydrogen or electron donation abilities of the consistent plant extract and some other clean components (DPPH). In this spectrophotometric research, stable radicle DPPH is used as a reactant. 50 mm of 0.004% methyl alcohol solution 01 to 05 mm of diverse amounts of the methyl alcohol extract were added (DPPH). Put in incubator for half hour. Period at room temperature. Against a blank at 517nm the absorption was read. By using the mention below formula, the free radical DPPH was calculated

$$\text{DPPH scavenging activity \%} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where,

A_{blank} =Absorbance of the control

A_{sample}=Absorbance of the test compound

Concentrations of extract providing 50% inhibition (IC_{50}) were considered from the graph plotted percentage inhibition in contrast to the concentration of extract. Vitamin C namely Ascorbic acid was used as a positive control protocol with little alteration [18].

Experiment was repeated three times.

RESULTS

1. Qualitative phytochemicals screening of *Quercus glauca*

There are number of primary and secondary metabolites were present in the back of above mention plant. In all extracts, tannins, phenol, alkaloids, carbohydrates, phlobatannins, cardiac glycoside were present. In distilled water, terpenoid was present while absent in both of methanolic and ethanolic extract. Presence of Saponins, glycosides, courmine, anthraquines, flavonoids and oil was detected (Table 2).

Table 2. Qualitative phytochemical analysis of bark

S.No.2	Phytochemical test	Plant extract		
		Ethyl Alcohol	Methyl Alcohol	Distilled water
1.	Tannins	+	+	+
2.	Saponins	+	-	-
3.	Phenol	+	+	+
4.	Terpeniod	+	+	-
5.	Flavonoids	-	-	-
6.	Glycoside	-	-	-
7.	Quinones	+	+	-
8.	Courmine	-	-	-
9.	Alkaloid	+	+	+
10.	Carbohydrate	+	+	+
11.	Phlobatannins	+	+	+
12.	Anthraquinones	-	-	-
13.	Cardiac Glycoside	+	+	+
14.	0il	-	-	-

(+) Existent (–) Non Existent

2. Antibacterial activity

The aim of this study was to know how methanolic crude extract of *Quercus glauca* bark was usefull verses five different strains of bacteria. In methanolic extract of bark, the maximum zone was noted against *K.pneumoniae* and *A.baumanii* (13.33 mm). The smallest zone was noted around *Pseudomonas aeruginosa* (10.33 mm). For antibiotic activity, (27mm) maximum zone was recorded against *P.aeruginosa* and that of (11.3mm) against *Klebsella pneumonia.* As a negative control, no zone of inhibition exhibited by DMSO verses any bacterial strains. Different inhibition zones showed by the extract of bark verses various strains of bacteris. So it is concluded that the bark has the abiliry to be utilize to treat numbers of illnesses (Figure 2).

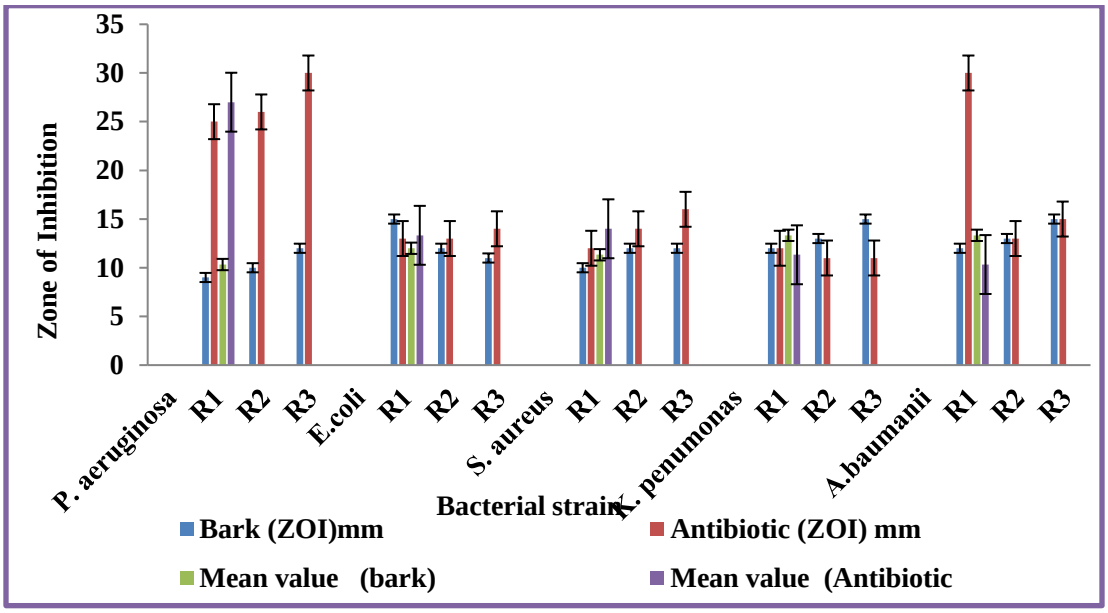

Fig 2: Graphical representation of the bark extract of *Quercus glauca* Thunb.

3. Antifungal Activity

To know the antifungal activity of crude extract of the bark there were three kinds of different fungal strains were used. Against *Trichophyton mentagrophytes*,the maximum zone (15mm) was recorded and that of minimum zone against *M.citri*, (12.66mm) was noted. For antibiotic, gainst *M.citri*, (25.33mm) there was a maximum zone and against the *Penicillium* there was a minimum (15.66) mm zone was recorded. So, it is resulted that the bark of this plant has antifungal ability (Figure 3).

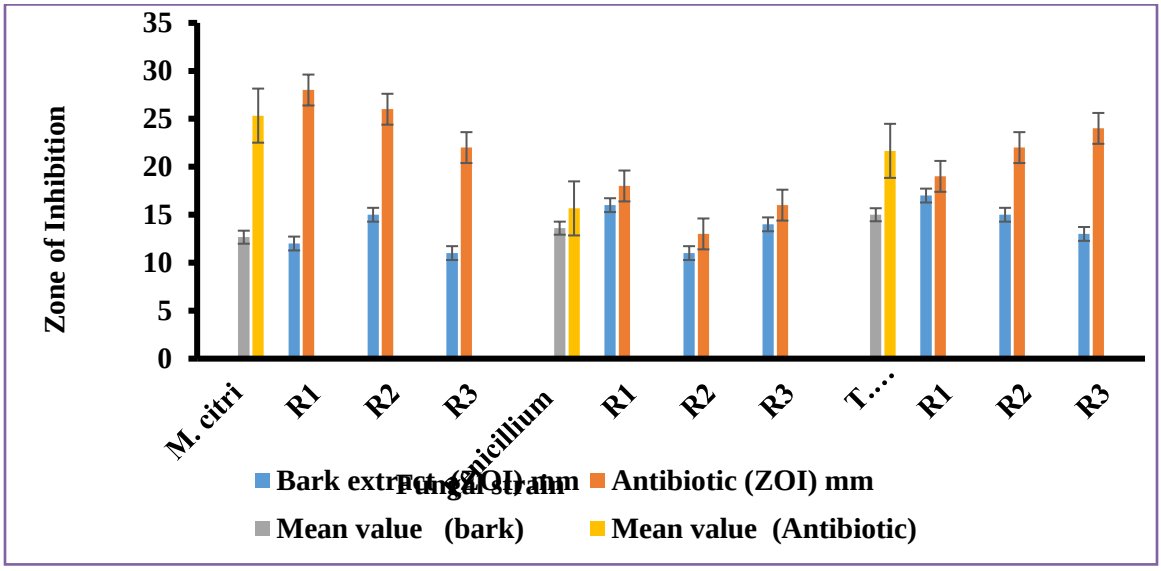

Fig 3: Graphical representation of the bark of Qurcus glauca Thunb.

4. Cytotoxic activity

Against Brine shrimp larvae (nauplii), the above mentioned activity was checked. 10µg/ml, 100µg/ml and 1000µ/ml these were the three concentrations of extract that were used. The quantity of dead nauplii was enumerated. 07 nauplii were dead at 10µg/ml, 09 at 100µg/ml with the percentage of 90%. 10 nauplii were dead at 1000µg/ml with the death percentage of 100%. Conclusion showed the cytotoxicity of the plant (Figure 4).

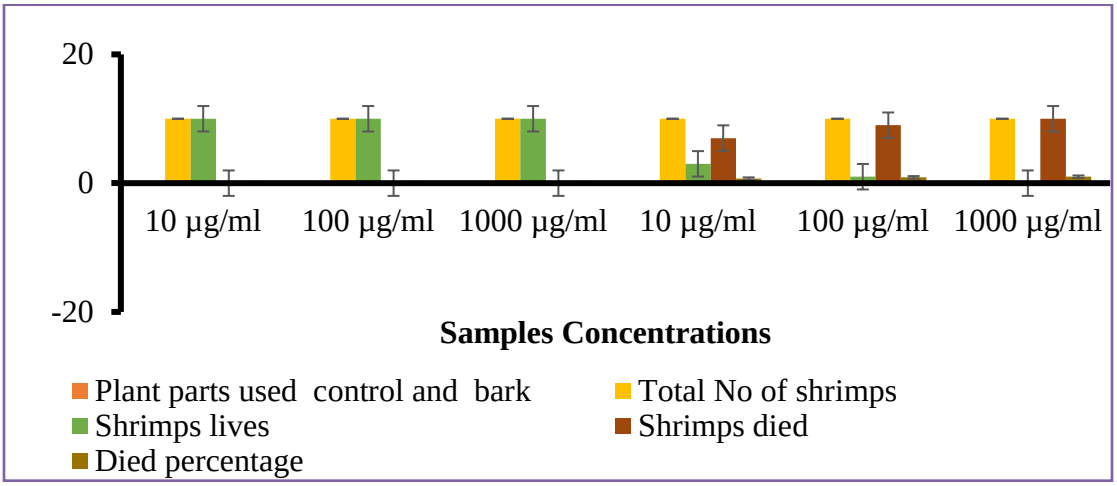

Fig 4: Graphical representation of cytotoxic activity of bark of *Quercus glauca* Thunb.

5. Phytotoxic Activity

Bark extract showed the significant results against *Lemna minor* L. At 10µl the growth inhibition was 8.66%, at 100µl that was 28.40% and 84.70% at 1000µl. The phytotoxicity of the bark showed that the plant can be used in upcoming times as a weedicide (Figure 5)

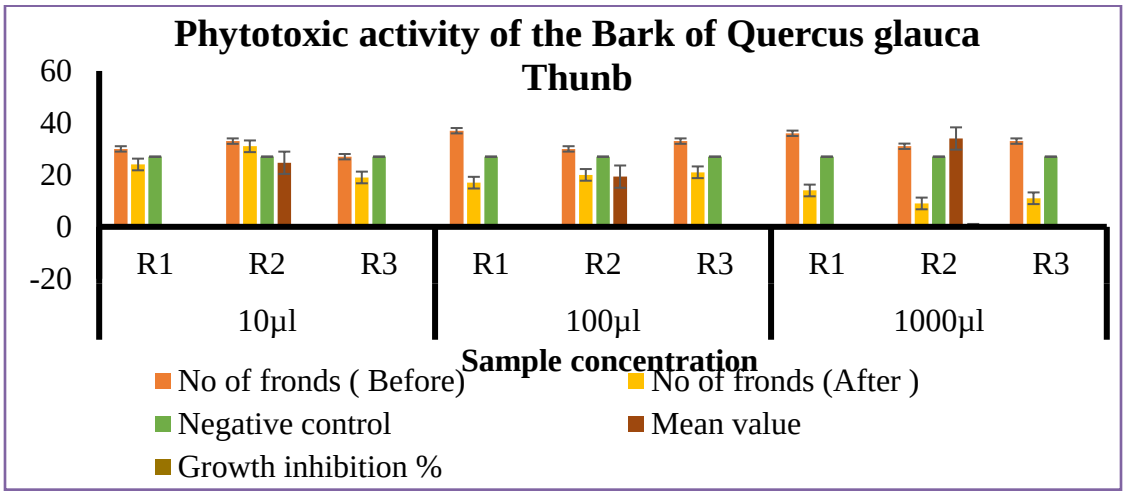

Fig 5: Phytotoxic activity of the bark of *Quercus glauca* Thunb.

6. Antioxidant potential

By using DPPH, the above mentioned activity was checked. In various concentrations the inhibiton was evaluated such as 1000µg/ml, 500µgml, 250 µg/ml and 100µg/ml. At all concentrations, the color of DPPH was decolorized. So the decolonization shows that the bark have antioxidant potential (Figure 6).

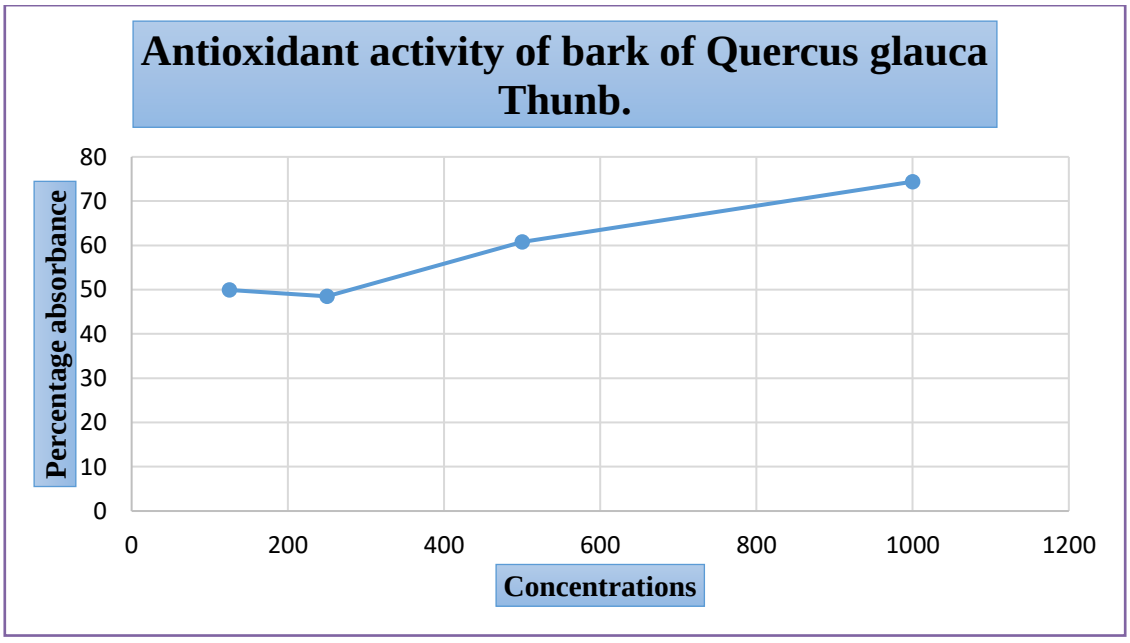

Fig 6: Graphical representation of *Quercus glauca* Thunb.

DISCUSSION

All around the world, people use medicinal plants and all those medicines that derived from plants. So they are becoming well known rapidly in advance communities as natural addition to artificial drugs [19]. *Quercus glauca* Thunb. of family Fagaceae and has a lot of therapeutic possessions. According to my information, in pharmacognosy, not so much work has been done so far on the bark of this plant. So this study was performed on the bark of *Quercus glauca* micro and macroscopic features, powder drug, examination of phytochemicals and different biological acivities like antimicrobial, cytotoxicity, phytotoxicity and antioxidant potential [20]. For phytochemical screening, the bark extract was used. The extract was made ready with ethyl alcohol, methyl alcohol and aqueous water [21]. It was concluded that secondary metabolites like tannins, carbohydrates, saponins, phenols, phlobatanins, and quinones were detected. While oil, courmine, and anthraquinones were not detected. By the presence of these metabolites it was confirmed that the plant has medicinal characteristics [22]. Against different strains of bacteria (included gram negative and gram positive) the methanolic extract was used. This research concluded that the bark of plant has antibacterial ability [23]. Against Acinetobacter *baumanii* and *Klebsiella pneumoniae* there was recorded largest zone (13.33) mm and that of smallest zone of against *P.aeruginosa* (10.3) mm. So in antibiotic, the biggest inhibition zone was noted against *P.aeruginosa* (27) mm and that of minimum zone against *K.pneumoniae* (11.33) mm. It is resulted that the bark have antibacterial ability. Against three different strains of fungus, the methanolic extract of bark was tested [24]. Largest zone of inhibition was showed against *Trichophyton mentagrophytes* (15 mm) while smallest zone was noted against *Mycospharella citri* (12.66 mm). In antibiotic, *M.citri* showed largest zone (25.33mm) and *Penicillium* showed the smallest zone (15.66 mm). The cytotoxicity of bark extract was tested through the Brine shrimp lethality assay (BSLA) [25]. Consequential cytotoxicity was shown at all concentrations. All the napulii were dead at 1000µg/ml. So the result concluded that the bark of this plant have cytotoxic capability. Against *lemna minor* L. the phytotoxicity of the plant was checked. At the concentration of 1000µl the methanolic extract exhibited the highest growth inhibition, which was 87.40%. Result of phytotoxicity, the bark of the plant ability to kill and undesirable weeds so the crops may be rapidly grow. The antioxidant free radical scavenging action of bark exhibited that the plant has antioxidant ability [26].

CONCLUSION

Phytochemical screening of the bark indicates the presence of secondary metabolites like tannins, phenol, saponins, carbohydrates, cardiac glycosides, and terpenoids. The present study provided novel scientific information about the bark of *Quercus glauca* and its phytochemical assay and biological potential that has certainly not been recorded previously, showing promising antioxidant, phytotoxic, cytotoxic, antifungal and antibacterial activities. The outcomes of the present research suggest the use of the bark of *Q.glauca* in the discovery of medicines for the prevention of various infections and diseases.

REFRENCES

1. Al Hawani, I. A., Adhere, A. H., & Abdalzahra, I. M. (2020). A Biological Study on *Quercus* Bark as Antimicrobial Agent. *Annals of Tropical Medicine and Health*, *23*, 23-119.
2. Zafar, S., Khan, A., Parveen, Z., Khan, M., & Khan, K. (2014). In vitro Evaluation of Anti-Microbial Potential of the Leaf Extracts *of Acacia modesta*. *Journal of the Chemical Society of Pakistan*, *36*(6).
3. Ahmad, M., Zafar, M., & Sultana, S. (2009). *Salvadora persica*, *Tamarix aphylla* and *Zizyphus mauritiana*-Three woody plant species mentioned in Holy Quran and Ahadith and their ethnobotanical uses in north western part (DI Khan) of Pakistan. *Pakistan Journal of Nutrition*, *8*(5), 542-547.
4. Masika, P. J., & Afolayan, A. J. (2003). An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa. *Pharmaceutical Biology*, *41*(1), 16-21.
5. Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M, Ozbek T (2006). Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii Afan*. (Asteraceae). Turk. J. Biol. 30: 65-73
6. Khodadoust, S., Mohammadzadeh, A., Mohammadi, J., Irajie, C., & Ramezani, M. (2014). Identification and determination of the fatty acid composition of Quercus brantii growing in southwestern Iran by GC–MS. *Natural product research*, *28*(8), 573-576.
7. Gupta, S. S. (1994). Prospects and perspectives of natural plant products in medicine. *Indian J Pharmacol*, *26*(1), 1-12.

8. Jamil, M., ul Haq, I., Mirza, B., & Qayyum, M. (2012). Isolation of antibacterial compounds from *Quercus dilatata* L. through bioassay guided fractionation. *Annals of Clinical Microbiology and Antimicrobials*, *11*(1), 1-11.
9. Abbas, S. A., Nighat, F., Ahmed, G., Choudhary, M. I., Alvi, K. A., De Silva, K. T. D., & Arambewela, L. S. R. (1991). Chemical constituents of Alstonia macrophylla. *Journal of Natural Products*, *54*(3), 750-754.
10. Sofowora, A. (1993). Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria*, 134-156.
11. Adegoke, A. A., & Adebayo-Tayo, B. C. (2009). Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum*. *African Journal of biotechnology*, *8*(1).
12. Evans, W. C. (2009). *Trease and evans' pharmacognosy E-book*. Elsevier Health Sciences.
13. Roopashree, T. S., Dang, R., Rani, S. R. H., & Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis. International Journal of Applied research in Natural products*, *1*(3), 20-28.
14. Basri, D. F., Tan, L. S., Shafiei, Z., & Zin, N. M. (2012). In vitro antibacterial activity of galls of *Quercus infectoria* Olivier against oral pathogens. *Evidence-Based Complementary and Alternative Medicine*, *2012*.
15. Sarah, Q. S., Anny, F. C., & Mir, M. (2017). Brine shrimp lethality assay. *Bangladesh Journal of pharmacology*, *12*(2), 186-189.
16. B. Meyer, N. Ferrigni, J. Putnam, L. Jacobsen, D. Nichols, and J. L. McLaughlin, "Brine shrimp: a convenient general bioassay for active plant constituents," *Planta med,* vol. 45, pp. 31-34, 1982.
17. Khan,S. A., and Ibrar, M.2017. Cytotoxic and phytotoxic Activities of Reinwardtia trigyna (ROXB).International journal of Applied Agricultural Sciences, 3(2), 32.
18. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

19. Cruz MC, Santos PO, Barbosa AM Jr, et al. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol 2007; 111(2): 409-12.
20. Zampini IC, Cuello S, Alberto MR, et al. Antimicrobial activity of selected plant species from "The Ar-gentine Puna" against sensitive and multi-resistant bacteria. J Ethnopharmacol 2009; 124(3): 499-505.
21. N.I. Paphitou, 'Antimicrobial resistance: Action to combat the rising microbial challenges', Int. J. Antimicrob. Agents 2013, 42, 25 – 28.
22. Rios, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100, 80-84.
23. N.I. Paphitou, 'Antimicrobial resistance: Action to combat the rising microbial challenges', Int. J. Antimicrob. Agents 2013, 42, 25 – 28.
24. Rios, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100, 80-84.
25. Sule WF, Okonko IO, Joseph TA, et al. In-vitro Antifungal Activity of Senna alata L. Crude leaf extract. Res J Biol Sci. 2010;5(3):275-84.
26. Timothy SY, Lamu FW, Rhoda AS, et al. Acute toxicity, Phytochemistry and antibacterial activity of aqueous and ethanolic leaf extracts of Cassia alata L. Int Res J Pharma. 2012;3(6):73-6.

AUTHORS

First Author: Meryam Iqbal, MPhil scholar, Department of Botany Hazara University, Mansehra, KP, Pakistan,

Second Author: Prof. Dr. Ghulam Mujtaba Shah, Chairman, Department of Botany, Hazara University, Mansehra. 21300, Khyber Puhtunkhwa, Pakistan,

Third Author: Dr. Muhammad Fiaz, Assistant Prof, Department of Botany, Hazara University, Mansehra, 21300, Khyber Pakhtunkhwa, Pakistan,

Fourth Author: Khursheed ur Rahman is currently pursuing Ph.D degree from Botany Department, Hazara University, Mansehra, KP, Pakistan,

Fifth Author: Samia Abbasi, MPhil scholar, Department of Botany Hazara University, Mansehra, KP, Pakistan,

Sixth Author: Zain Ul Abdin, Ph.D scholar, Department of Botany Hazara University, Mansehra, KP, Pakistan, ,

Seventh Author: Iqra, MPhil scholar, Department of Botany Hazara University, Mansehra, KP, Pakistan, ,

Eight Author: Zabeeh Ullah, MPhil scholar, Department of Botany Hazara University, Mansehra, KP, Pakistan,