Makara Journal of Science

Volume 26
Issue 2 *June*

Article 5

6-30-2022

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Makara Journal of Science, 26/2 (2022), 114–119
doi: 10.7454/mss.v26i2.1351

Phytochemical, Antibacterial, Antifungal, and Hemagglutination Screening of *Quercus agrifolia Nee* Root Extracts

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Received April 16, 2022 | Accepted June 30, 2022

Abstract

In the current study, the roots of *Quercus agrifolia Nee* were subjected to phytochemical analysis to determine the presence of various compound groups. To explore the effect of *Quercus agrifolia Nee* roots, we evaluated four solvent extracts, namely, crude ethanolic extract (CEE), *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc), for their antibacterial, antifungal, and hemagglutination activities. Agar well diffusion and agar tube dilution methods were used to determine the antibacterial and antifungal effects, respectively, and hemagglutination activity was measured against human erythrocyte blood groups. The qualitative phytochemical analysis of the solvent extracts of *Quercus agrifolia Nee* roots showed the presence of different classes of compounds, i.e., flavonoids, coumarins, terpenoids, etc. The CEE and various fractions were tested against *Escherichia coli*, *Listeria monocytogenes*, *Alcaligenes faecalis*, and *Pseudomonas aeruginosa*, with CEE exhibiting the widest zone of inhibition against *A. faecalis* (30 mm) and moderate activities against *P. aeruginosa* (18 mm%), *L. monocytogenes* (18 mm%), and *E. coli* (20 mm%). The *n*-hexane fraction and the remaining fractions had moderate to good activity against all the four bacterial strains. The antifungal ability of plant roots was also tested against four separate fungal strains, i.e., *Alternaria solani*, *Aspergillus niger*, *Triticum harzianum*, and *Fusarium oxysporium*, and the *n*-hexane extract showed a higher activity than the CEE. During the investigation by hemagglutination assay, some solvent extracts exhibited a low amount of lectin complex in the selected plant. The current study revealed that the roots of *Quercus agrifolia Nee* has an ideal medicinal viability and contains a diverse range of phytochemical classes. This research is the first systematic study of *Quercus agrifolia Nee* roots, demonstrating its versatility in a variety of biological activities.

Keywords: antibacterial, antifungal, haemagglutination, phytochemical screening, Quercus agrifolia Nee roots

Introduction

Medicinal plants have piqued the interest of researchers. The main reason for this curiosity is the discovery of various groups of compounds and their possible use in the treatment of chronic illnesses [1–3]. Medicinal plants have a long history of use as medicine in the Indian subcontinent, and the use of herbal medicine for medicinal purposes is gaining popularity worldwide [4, 5]. For the treatment of various infectious diseases, a large portion of the world's population, especially in developing countries [6], uses the traditional medical system. In general, plant groups are used in herbal medicine, and they are an important source of potent and effective drugs in tropical countries for the treatment of different illnesses identified with cerebral pain, stomachaches, wounds, etc. [7]. In the pharmaceutical industry, South Asian countries use 8000 medicinal plants to cure different diseases, with Pakistan accounting for 19% of these plants [8].

Plant-based medications are used all over the world, with about 80% to 85% of them being used in developing countries and a quarter of the population in the United States [9]. According to the World Health Organization, approximately 60,000 to 70,000 medicinal plants are used in conventional drug systems around the world [10]. Pakistan's flora is composed of dense hilly and woodlands, and a variety of therapeutic plants found in various parts of the country are widely used locally for medicinal purposes [11].

Approximately 600 species belong to the genus *Quercus*, and one of them is *Quercus agrifolia*, also known as California live oak or coastal live oak. This plant is a dense shrubby tree with several nodes and reaches a height of 11–26 m. *Quercus agrifolia* can be found in hard and hilly areas all over the world, from Mexico to Nevada in America and most notably in China and Central Asia's mountainous regions [12, 13]. The medicinal and therapeutic properties of species belonging to "*Quercus*" are diverse. Various parts of different species are used to treat digestive diseases and gonorrhea, mainly in babies. In humans, *Q. agrifolia* is used to treat diarrhea and asthma and acts as a diuretic, possessing a strong antibacterial activity

against different bacterial strains [14]. *Quercus* (Fagaceae) have many applications in humans and animals, especially in regard to food as it plays a role in wine maturation in oak barrels. Oak is also used in the wood industry especially for wood coloring and is used to protect wood from fungal decay [10 ,11]. According to a study [13], certain oak species also have antifungal, anti-diarrheic, and astringent effects, which are helpful in the treatment of the inflammation of oral and anal mucosa and used in wound healing. *Quercus* species contain high contents of saponins and phenolic compounds, such as tannins, flavonoids, and pro-anthocyanidins, which have anti-microbial, anti-inflammatory, anti-oxidant, and anti-tumoral properties [12, 13].

This research aimed to use plant extracts for phytochemical screening and biological investigation. Despite the numerous pharmacological studies conducted on many *Quercus* species to date, not every species has been thoroughly examined. According to our review of the literature, no research has been performed on the phytochemical, hemagglutination, antibacterial, or antifungal properties of *Quercus agrifolia Nee* roots. We report for the first time the above biological activities of several solvent system extracts obtained from *Quercus agrifolia Nee* roots.

Materials and Methods

Collection of plant material. The roots of *Quercus agrifolia Nee* were collected in South Waziristan's hilly zone, the southern part of Waziristan and Pakistan's Khyber Pakhtunkhwa merged district. Plant specimens were verified at the University of Peshawar, Department of Botany. The voucher specimen has been stored in the department's herbarium under the number: QANR 1085.

Preparation and fractionation of crude extract. The air-dried roots of *Q. agrifolia Nee* were powdered and macerated with ethanol at room temperature for one week to produce the crude solvent extract. The solvent was extracted using a rotary evaporator after maceration to obtain the crude ethanolic extract (CEE). The final crude extract was suspended in 400 mL distilled water. Different polarity-based solvents, such as *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc), were used to separate the suspension into different fractions [15].

Preliminary phytochemical analysis. The presence of various secondary metabolites was qualitatively investigated in each extract of the plant roots through already established methods [15, 16].

Test for alkaloids: Mayer's test. The extract was mixed and heated with 5 mL HCl solution (2 N). The solution was then cooled, filtered, and added with Mayer's reagent (1 mL). The existence of alkaloids was indicated by the appearance of turbidity or precipitation.

Test for saponins: Foam test. A total of 2 mL filtrate was combined with 5 mL distilled water and vigorously shaken for 2 min. The presence of saponins was shown by froth formation.

Test for tannins: $FeCl_3$ test. The test solution consisted of 2 mL distilled H_2O and a few drops of ferric chloride. The formation of brown or bluish-black coloration indicated the presence of tannins.

Test for flavonoids: Alkaline reagent test. In 2 mL 10% NaOH, approximately 0.2 g extract was dissolved. After the addition of 0.5 mL HCl, the color of the solution changed from yellow to colorless, indicating the existence of flavonoids.

Test for steroids: Chloroform test. In a test tube, 0.2 g extract was treated with 2 mL $CHCl_3$ solvent. A total of 1 mL H_2SO_4 conc. was applied to this mixture. The appearance of red color in the $CHCl_3$ layer proved the presence of steroids.

Test for terpenoids: Salkowski test. About 0.2 g sample was carefully dissolved with 2 mL $CHCl_3$ and 3 mL conc. hydrochloric acid to form a layer. The formation of reddish-brown coloration at the interface showed the existence of terpenoids.

Test for tannins: Precipitate test. The presence of tannins was demonstrated by the formation of a red precipitate after boiling 2 mL extract with 2 mL HCl (1%).

Test for coumarins: NaOH test. In a test tube, 2 mL extract was mixed with 3 mL 10% sodium hydroxide. Coumarins were present if the solution turned yellow.

Antibacterial activity. Using the agar well diffusion process, the extracts of *Quercus agrifolia Nee* roots were tested against *Escherichia coli*, *Listeria monocytogenes*, *Alcaligenes faecalis*, and *Pseudomonas aeruginosa* [17]. All the test bacteria were available at the laboratory of Gomal Center of Biochemistry and Biotechnology, Gomal University. To create a bacterial lawn, we inoculated 18 h-old nutrient-broth culture to nutrient-agar plates. A sterile borer (6 mm) was used to render wells in the agar-containing plates. The extract (3 mg) was combined with dimethyl sulfoxide (DMSO) (1 mL) as a stock solution to prepare the test samples. A total of 100 µL of each stock solution was poured into each well and incubated at 37 °C for 24 h. The positive control was amoxicillin, and the negative control was DMSO. The percentage zone of inhibition of the bacterial strains was calculated in millimeters in comparison with the positive control.

Antifungal activity. The agar tube dilution method was used for antifungal activity against *Alternaria solani*,

Aspergillus niger, *Triticum harzianum*, and *Fusarium oxysporium* [5]. The stock solutions for the various plant extracts were prepared in 1% DMSO at a concentration of 24 mg/mL. For the growth of fungi, Sabouraud-dextrose agar medium was prepared. After cooling to about 40 °C–45 °C, the medium was transferred to test tubes (4 mL), and plant extracts (66.66 mL) were added. For sterility testing, the test tubes were placed in a slanting position to solidify the contents and incubated overnight at 28 °C±1 °C. Then, using a flame-sterilized inoculating loop, 5–7-day-old fungal strains culture were inoculated into the test tubes. The positive control was miconazole, and the negative control was DMSO. The percent inhibition was determined using the following formula:

Percent Inhibition = 100 − Linear growth in test (mm)/Linear growth in Control (mm) × 100

Hemagglutination activity. Hemagglutination assay was tested against all human ABO blood groups [18]. The stock solution for the test samples was prepared at a final concentration of 1 mg/mL, and each sample was serially diluted. Using disposable syringes, blood was drawn from healthy people and placed in an ethylenediaminetetraacetic acid tube. The erythrocytes were then isolated after centrifugation. A 2% RBC suspension was set up in phosphate-buffered saline (pH 7.4) of all blood types. The test sample dilution (1 mL) was then incubated at 25 °C when combined with 1 mL 2% RBCs. The results were reported after the incubation period. A negative activity was indicated by smooth-button formation at the bottom, and positive activity was denoted by rough granular deposition at the bottom. The degree of deposition was used to evaluate the presence of hemagglutination.

Minimum inhibition concentration. Plant extracts were obtained at concentrations ranging between 10–50 mg/mL and all poured in test tubes. Then, each test tube was poured with fresh culture of all bacterial strains and diluted to determine the final concentration. After the procedure, all the test tubes were placed in an incubator to give a suitable environment for growth. The minimum amounts of extracts at which no growth was observed were regarded as the minimum inhibition [4].

Results and Discussion

Preliminary phytochemical screening. The qualitative phytochemical analysis of solvent extracts of *Q. agrifolia Nee* roots showed the presence of different classes of compounds. The existence of these various groups of chemical compounds may explain the biological activities of this plant.

Table 1 shows that CEE, EtOAc, and $CHCl_3$ fractions contained alkaloids, flavonoids, and saponins, and *n*-hexane fractions comprised flavonoids only. Steroids and terpenoids were found in good amounts in the CEE and $CHCl_3$ fractions. Coumarins were present in all fractions but in greater amounts than CEE and EtOAc fractions. Tannins were detected in CEE and $CHCl_3$ fractions only. Except for EtOAc fraction, tannins were not detected in all fractions. These findings are in accordance with previous *Quercus* species records [19–21].

Antibacterial assay. The extracts used in this study displayed various zones of inhibition against bacterial populations (Figure 1). According to the findings, CEE exhibited the largest inhibition zone against *A. faecalis* (30 mm) and moderate activities against *P. aeruginosa* (18 mm), *L. monocytogenes* (18 mm), and *E. coli* (20 mm). The *n*-hexane fraction showed a low activity against *P. aeruginosa* (6 mm) and a moderate activity against *L. monocytogenes* (21 mm), *A. faecalis* (20 mm), and *E. coli* (14 mm). $CHCl_3$ fraction displayed a good activity against *P. aeruginosa* (22 mm) and was moderately active against *A. faecalis* (25 mm), *E. coli* (12 mm), and *L. monocytogenes* (12 mm). Moderate activity was shown by the EtOAc fraction against *L. monocytogenes* (21 mm), *P. aeruginosa* (16 mm), *A. faecalis* (18 mm), and *E. coli* (26 mm). The results showed that CEE and its different fractions had significant antibacterial activities against all of the bacteria tested. Bacterial species cause a wide range of diseases, and studies have shown that they are

Table 1. Qualitative Screening of Phytochemicals in *Quercus agrifolia Nee* Roots

Phytochemicals	Extract/Fractions			
	Ethanol	*n*-hexane	Chloroform	Ethyl acetate
Alkaloids	+	-	+	++
Flavonoids	+++	+	++	+++
Saponins	+	-	++	+
Tannins	-	-	-	+
Steroids	+++	++	-	+
Terpenoids	+	+	+++	-
Tannins	+	-	+	-
Coumarins	++	+	+	++

Phytochemicals: +++ profoundly present; ++ moderately present; + slightly present; - absent

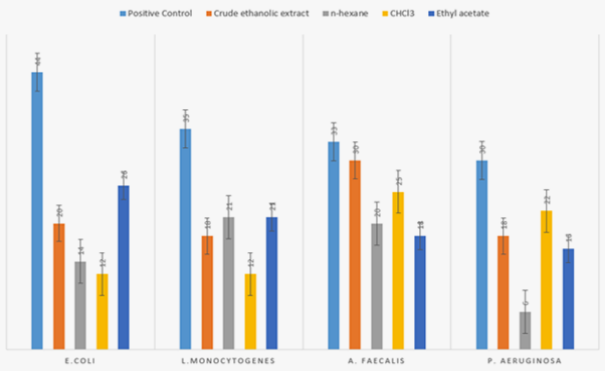

Figure 1. Antibacterial Properties of *Quercus agrifolia Nee* Root Extracts

resistant to a wide range of medications. Penicillin G, tetracyclines, gentamicin, and macrolides are among the antibiotics to which bacterial strains have developed resistance [22]. Previous research on *Quercus (Quercus brantti* and *Quercus presice)* has shown their antibacterial activity, but the mode of action and efficiency must be investigated further [23].

Minimum inhibitory concentration (MIC). MIC_{50} is the chemical concentration that inhibits 50% visual growth of any bacterial species. The lower the value of MIC, the more potent an extract is against a bacterial strain. The samples were screened to check the MIC_{50} against different bacteria [24]. The lowest MIC50 value was 1.3 mg/mL for the EtOAc fraction against *P. aeruginosa*, and the highest was 2.4 mg/mL for some strains displayed by CEE, $CHCl_3$, and *n*-hexane fractions. The rest of the MIC50 values varied among bacterial strains. Table 2 summarizes the results of MIC value.

Antifungal activity. Fungi produce mycotoxins or allergens and are a significant cause of allergies and a variety of other problems in humans. Synthetic fungicides are toxic to humans, and pathogens are developing resistance to them [25]. According to research reports, extracts from the leaves, stems, and roots of some medicinal plants have been used as antibacterial and antifungal [26, 27].

Using the agar tube dilution process, the antifungal activity of the CEE and various fractions of *Q. agrifolia Nee* roots was tested against four fungal strains. Our analysis (Figure 2) indicated that the *n*-hexane fraction showed the highest activity against all fungal strains (*A. solani* (68%), *T. harzianum* (66%), *A. niger* (56%), and *F. oxysporum* (50%)) compared with the other extracts. The antifungal activity was moderate for the CEE (*A. solani*, *T. harzianum*, *A. niger*, and *F. oxysporum* with percentage inhibitions of 52%, 52%, 47%, and 46%, respectively). Further, the $CHCl_3$ and EtOAc fractions showed no inhibitory activity against all fungal strains. The presented results possibly indicate that the tested extracts have different levels of activity for each fungal strain studied.

Hemagglutination activity. Lectins are found in many plants, and they have become a reliable way to study various aspects of cancer and metastasis. Lectins may be used for tumor cell identification, host immune defense augmentation, mitogenic enhancement, cytotoxicity, apoptosis, RBC agglutinations for ABO blood group determination, etc. [28, 29]. Some herbal plants have hemagglutination activity in certain blood groups [30, 31].

Given the importance of lectin as mentioned above, CEE and various fractions of *Quercus agrifolia Nee* roots were tested for potential hemagglutination activity against all blood groups. The results indicated that at various dilutions, *n*-hexane, $CHCl_3$, and EtOAc showed poor responses to A positive, AB positive, and O positive blood groups, respectively. No evidence of hemagg-lutination caused by any of the extracts was found against the remaining blood types. Table 3 summarizes the findings.

Table 2. MIC_{50} Values of CEE and Various Fractions of *Q. agrifolia* Roots

Bacterial strains	CEE	$CHCl_3$	*n-hexane*	EtOAc
Listeria monocytogenes	2.0	2.0	2.0	1.7
Escherichia coli	2.0	2.4	2.4	1.8
Alcaligenes faecalis	2.0	2.0	1.6	1.5
Pseudomonas aeruginosa	2.4	2.4	2.0	1.3

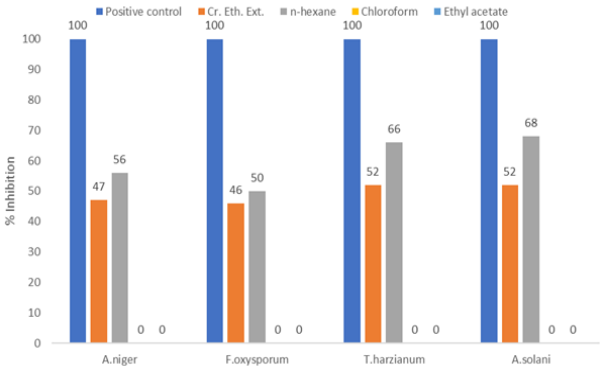

Figure 2. Antifungal Activity of *Quercus agrifolia Nee* Root Extracts

Table 3. Hemagglutination Assay of the Solvent Extract of *Quercus agrifolia Nee* Roots

Blood Groups	Crude Ethanolic Extract				*n*-Hexane				Chloroform				Ethyl acetate			
	1:2	1:4	1:8	1:16	1:1	1:4	1:8	1:16	1:1	1:4	1:8	1:16	1:1	1:4	1:8	1:16
AB^+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
AB^-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A^+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
A^-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B^+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B^-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
O^+	–		–	–	–	–	–	–	–	–	–	–	+	–	–	
O^-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

++ Strong agglutination; + weak agglutination; − no agglutination

Conclusion

A comparative evaluation of phytochemicals in the CEE and other fractions suggested that *Quercus agrifolia Nee*

roots are a rich source of biologically active phyto-constituents. All compared samples exhibited moderate to high antibacterial and antifungal potential. Although this plant has shown weak hemagglutination activity, further studies are needed to identify the main reason. The findings suggest that this plant can be explored for the preparation of antibacterial and antifungal drugs for pharmaceutical industries because all the extracts showed moderate to high activity against the different strains of bacteria and fungi. These antibacterial and antifungal activities can be due to the presence of secondary metabolites, which exhibit antibacterial and antifungal modes of action. However, further detailed studies are required to identify the phytoconstituents responsible for these bioactivities and evaluate the safety and efficacy of this plant.

Acknowledgments

The authors gratefully acknowledge Gomal Center of Biochemistry and Biotechnology, Gomal University D.I. Khan, KPK, Pakistan, for providing the laboratory facility for this research work.

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