Research Article

Phytochemical Analysis and Biological Evaluation of Methanolic Leaf Extract of *Corylus jacquemontii* Decne

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Abstract | There is a great concern for healthcare community in antibiotic resistance and evolution of novel bacterial strains. Numerous plants act as a source of novel compounds that could be used to develop new drugs. Therefore, the current study was aimed to investigate the methanolic extract of *Corylus jacquemontii* for phytochemical, antibacterial, antioxidant potential. The methanolic fraction of *C. jacquemontii* was evaluated for quantitative phytochemical constituents. The antibacterial efficacy was investigated via well diffusion method by using a concentration of 100 µg/ml. Furthermore, the antioxidant potential of extract was estimated at various concentration (50, 100, 150, 200, 250 µg/ml) through 2, 2-Diphenyl -1- picryl-hydrazyl (DPPH) assay (150 µg/ml). Results showed that the methanolic extract of *C. jacquemontii* is a rich source of many phytochemically active compounds such as alkaloids content (3.60%), flavonoids (3.40%) and terpenoids 2.30%. The methanolic extract of *C. jacquemontii* depicted promising antibacterial potential towards various tested bacterial strain in order of *P. aeruginosa* > *E. coli* > *S. typhi* > *MRSA* comparable to vancomycin. The highest percent mean growth inhibition (MGI) was observed against *P. aeruginosa of* 90.5±4.3 compared to vancomycin (61.26±6.31). The Zone of Inhibition (ZOI) noted against *P. aeruginosa* was 20 mm compared to vancomycin (17 mm). The quantitative test antioxidant activity of the methanolic fraction of *C. jacquemontii* at 250 µg/ml revealed the best antioxidant potential when the DPPH concentration of 150 µg/ml was used. The result showed that extract had a significant effect on antioxidant activity. Therefore, the present study supports the effectiveness of methanolic leaf extract of *C. jacquemontii* in traditional medicine and may be investigated further for mode of action on molecular basis.

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Introduction

In last 30 years antimicrobial agents had played an extremely vital role in management of many deadly infectious diseases (Nasrullah *et al*., 2012), but the emergence of microbial resistance and evolution of different strains have reduced their efficacy (Zaman *et al*., 2017). Infections caused by these resistant strains have serious consequences such as increased hospitalization, higher cost and escalated mortality and morbidity rates. According to Centre for Disease Control (CDC), resistant bacteria infect 2 million people in United States per year and it was estimated that direct health care cost was $20 billion and loss in productivity was $35 billion (CDC, 2013). The consequences were more drastic in low-income countries where people lack efficient surveillance and diagnostics. They cannot afford antimicrobials because of their limited income. It was estimated that if no fruitful efforts were made to intervene new drugs, death toll will reach to 10 million with cost of $100 trillion till 2050 in the world (Solomon and Oliver, 2014; Rather *et al*., 2017; Shehadeh *et al*., 2016; Courvalin, 2016; Morehead and Scarbrough, 2018). Therefore, the quest for effective and affordable antimicrobial agents were increasing day by day in health care especially in developing countries where deaths were mainly due to infectious diseases and resistance to many antibiotics (Awouafack *et al*., 2013; Srivastava *et al*., 2014; Theuretzbacher and Mouton, 2011; Walsh and Toleman, 2012). The natural resources such as plants are ultimately an important part to be screen for effective and novel drug development to tackle the problem of high cost and drug resistance.

The use of natural products and secondary metabolites originated from living system, mainly from plants had shown a boost to health care since ancient time. Furthermore, the modern medical science success rate is also dependent on the drugs that are acquired from natural resources. The limitless application of natural resources in treating many human diseases upsurge its usage due to its low cost and no side effects. WHO reported that nearly 65-80 percent population of the world rely on herbal medicine for treating different diseases (Gurinder and Daljit, 2009). Many studies have reported that plants contain bioactive compounds such as alkaloids, flavonoids, terpenoids and glycosides, that have a strong propensity for microbial agents (Obafemi *et al*., 2006). Plant extracts have previously been used on a variety of bacterial species, including *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and were found at various levels to be effective (Mostafa *et al*., 2018). Antibacterial activity of *Persea americana* against *Bacillus cereus* was observed at concentration of 10 and 25 mg/ml by using butanolic stem bark extracts (Akinpelu *et al*., 2015). Antimicrobial activity against twelve pathogenic microorganisms in four separate plant extracts were studied and found that most extracts could minimize the growth of pathogenic microorganisms (Manandhar *et al*., 2019).

C. jacquemontii (Decne), belongs to corylaceae family, and is commonly called Thangi or Thankoli. A deciduous tree with a height of 21m. The flowering stage begins from April to May, and from September to October. It is one of the important and economical species in the western Himalayan region. The species of this plant is found in various countries, including Pakistan, High above sea level in Afghanistan and in western Nepal at 1900-3000 m. This plant is also used to guard against inflammation, antioxidant, anticancer and cardiovascular. The seeds are also used as a fruit and the local collectors sell it on the market for economic purposes as it ripens (Bhalodia and Shukla, 2011). The Current study aims to evaluate phytochemical, antibacterial, antioxidant potential.

Materials and Methods

Study area

The current work was conducted in the Department of Botany, Hazara university Mansehra, Pakistan. The Department provide all the facilities for the current research work.

Plant material collection and authentication

C. jacquemontii was collected from District Kohistan Khyber Pakhtunkhwa, Pakistan. The plant was identified with the assistance of flora from Pakistan, taxonomists, and various pictorial guides. Voucher specimen No- 56094 were allotted to the collected plant and deposited in the Herbarium of Department of Botany Hazara University, Mansehra, KPK, Pakistan. Leaves of *C. jacquemontii* was washed meticulously with clean tap water for soil waste removal followed by surface sterilization with 1% perchloric acid and 70% ethanol and dry in the shade afterward. Dry plant material was crushed through electrical grinder into powder form and stored in

bottles for future experiments (Iftikhar *et al*., 2020).

Extraction

Hot continuous extraction or Soxhlet extraction method was used for the phytochemicals extraction from *C. jacquemontii leaves*. This technique involved by putting the crushed plant material in the Soxhlet apparatus' thimble chamber. The extraction solvent, Methanol was heated in the flask and vaporized into the sample thimble. It is then condensed in the condenser and dripped back. All the liquid content when reached to the siphon arm were emptied in flask and the process was repeated (Konga *et al*., 2017). Briefly, methanol (600ml) was used to pulverized and soaked 200g of plant material for 48 hours in Soxhlet apparatus. The extract was dried after being concentrated by evaporation at 70 °C for 8 hours via rotary evaporator (Sengul *et al*., 2009). Further fractionation was performed into n-hexane and chloroform and evaporated at 40°C with Rotary Evaporator (Rahman *et al*., 1997; Nisar *et al*., 2011).

Quantitative phytochemical screening

Quantitative test for alkaloids: Quantitative test for alkaloids was performed followed by the previously reported procedure with slight modification (Harborne, 1992). Briefly, 5g of leaves powdered was taken and 200ml of methanol was added to it and kept for 4 hours. Concentrated ammonium hydroxide was added to the mixture and filtered. Finally, the precipitate was weighted according to the given formula.

$$Total\ weight\ of\ alkaloids = (w1 - w2g\ ) / w3$$

$$alkaloids\ yield = (w2 - w1)\ X\ 100 / w3$$

w_2= Petri plate with alkaloids weight; w_3= Quantification of initial plant sample weight.

Quantitative test for flavonoids: Bohm and Kocipai-Abyazan (1994) protocol was used for the quantitative determination of flavonoids content in the methanolic extract of *C. jacquemontii*. Briefly, 10g leaves powder was taken in a beaker and 80% methanol (80mL) was added and the mixture was filtered. The process was repeated again and again until complete purity. Finally, the extract was transferred to petri dish for drying. The dry extract was then weighted according to the formula.

$$\text{Weight of total Flavonoids} = (w1 - w2g\ ) / w3$$

$$Yield\ of\ Flavonoids = (w2 - w1)\ X\ 100 / w3$$

w_2= Flavonoid's weight with crucible; w_3= Estimation of initial weight of plant sample.

Quantitative test for terpenoids: The quantitative analysis for terpenoids was performed according to Indumathi *et al*. (2014) with some modifications. Briefly, 10g of leaves powder was soaked in 9ml of methanol. The extract was then transferred to another vial for drying. The % of terpenoids were then calculated by the following formula.

$$Total\ \text{Terpenoids}\ weight = (w1 - w2g\ ) / w3$$

$$\text{Terpenoids}\ yield = (w2 - w1)\ X\ 100 / w3$$

w_2= weight of crucible with Terpenoids; w_3= initial weight of plant sample taking for estimations.

Determination of antibacterial activity

Test organisms: The different pathogenic bacterial strains used in the current study containing three Gram negative strains *Escherichia coli* (*E. coli*) (ATCC 25922), *Salmonella typhi* (ATCC 39183), *Pseudomonas aeruginosa* (ATCC 27853), and 1 Gram-positive strain *S. aureus* (ATCC 29213). These bacterial strains were cultured from original stock kept at -70°C. All the bacterial strains were provided by the center of biotechnology and microbiology, University of Peshawar and all the experiments were performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayne, 2014). Antibacterial activities of the extract were performed through broth dilution method (Stalons and Thornsberry, 1975) and well diffusion method (Wiegand *et al*., 2008).

Broth dilution method

Stalons and Thornsberry (1975), method was used to evaluate the antibacterial potential of methanolic extract of *C. jacquemontii* against the test microorganisms. Bacterial culture was prepared in Luria broth (LB) media and incubated for 12 h at 37 °C. After overnight of incubation the culture was diluted to 10^4 colony forming units (CFU) with fresh LB medium and methanolic extract (100 µg/ml) was added and plates were further incubated for 12 hr at 37°C. The treated and untreated cultures were then compared for growth. Vancomycin (30µg disc) was used as a standard control. The percentage mean

growth inhibition (MGI) was calculated by using the formula from three independent experiments.

$$\% MGI = [(dc - dt)/dc] \times 100$$

Well diffusion method

The prepared media (1000 ml distil water containing 28 g Nutrient agar) were sterilely poured onto the Petri plates. The homogenization of the bacterial culture was done in 8 ml broth medium (13 g Nutrient Broth/1000 ml distill water) in shaking water bath (37°C) for 16 hours at a speed of 200 rpm. After overnight of incubation the bacteria cultured were compared for turbidity (0.5 McFarland turbidity standards) and diluted accordingly. Bacteria culture was then spread on plate via glass spreader and kept at 37°C for setting of culture. With the help of cork borer wells were formed and 6µl of plant extract having concentration of 100 µg/ml was added to the wells. As a negative control, 10% dimethyl sulfoxide (DMSO) was used (Gonelimali *et al.*, 2018). Vancomycin (30 µg disc) was used as a standard. The minimal zone of inhibition (zoi) around each disc was measured in millimeters (mm) following 24 hours of incubation.

Antioxidant activity

A quantitative approach with certain modifications was used to test the extract's antioxidant properties (Sengul *et al.*, 2009). 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) was used, and 6.6 ml of it was dissolved in 15 ml tubes while they were wrapped in aluminum foil. The methanolic leaf extract of *C. jacquemontii* was obtained in separate tubes and combined with 1 mM of DPPH at various doses (50, 100, 150, 200, and 250 µg/ml). Each tube's color was checked, and the antioxidant activity of the extract was calculated based on the color changed.

Statistical analysis

The experimental data of antibacterial activity was analyzed by mean±SD from triplicate experiment by using GraphPad prism 8.1 software. * $p < 0.05$ was considered statistically significant calculated by student t-test (Student, 1908).

Results and Discussion

Quantitative phytochemical analysis

The presence of different phytochemical compounds/ secondary metabolites aided in plant antimicrobial activity due to its biologically active nature. The methanolic extract of *C. jacquemontii* revealed the presence of alkaloids (3.60%), flavonoids (3.40%) and terpenoids (2.30%) (Figure 1).

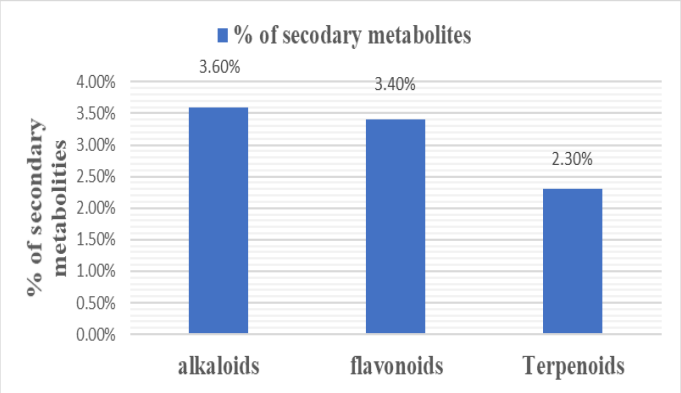

Figure 1: *Quantitative phytochemical analysis shows different secondary metabolites.*

Antibacterial potential of C. jacquemontii

Broth dilution method: The antibacterial potential of methanolic extract (100 µg/ml) of *C. jacquemontii* was performed on the test microorganism by accessing the % MGI with comparison to standard antibiotic vancomycin. The results showed that the leaves methanolic extract exhibited invitro bactericidal property. The growth of tested microorganism was hampered and the % inhibition was shown in Table 1 and Figure 2. The results showed % growth inhibition in order of *P. aeruginosa* > *E. coli* > *S. typhi* > *MRSA*.

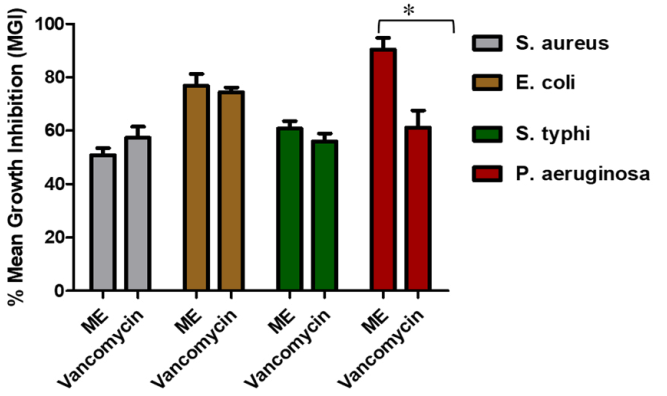

Figure 2: *Bar Graph of in vitro antibacterial potential of C. jacquemontii by broth dilution assay. Each bar represent % mean growth inhibition by methanolic extract against all tested bacterial strains compared to vancomycin (30 µg disc). Results were obtained from three independent experiment and displayed as mean±S.D. *p < 0.05 was considered statistically significant calculated by student t-test.*

Well diffusion method

Plant extracts have bioactive compounds or secondary metabolites which are linked to antibacterial activities. The methanolic extract of *C. jacquemontii* were further

Table 1: *% mean growth inhibition (% MGI) of various bacterial strains in the presence of methanolic leaves extract of C. jacquemontii at (100 µg/ml).*

Bacterial strains	Extract % MGI	Vancomycin (30 µg disc) %MGI
Pseudomonas aeruginosa (P.A) (ATCC 27853)	90.5±4.3	61.26±6.31
Escherichia coli (E. coli) (ATCC 25922)	76.86±4.4	74.5±1.7
Salmonella typhi (S.T) (ATCC 39183)	60.85±2.7	56.03±2.9
Methicillin resistant *Staphylococcus aurous* (MRSA) (ATCC 29213)	50.9± 2.6	57.4±4.17

tested against four pathogenic bacteria using disc diffusion assay. Based on ZOI production, *C. jacquemontii* methanolic extract at 100 µg/ml demonstrated very efficient antibacterial activity. The antibacterial activities depicted by the extract showed a ZOI from 15-20 mm against tested bacterial strain. The results were summarized in the Figure 3 and Table 2. The maximum inhibitory activity of the extract against bacteria is in the order of *P. aeruginosa* > *E. coli* > *S. typhi* > *MRSA*.

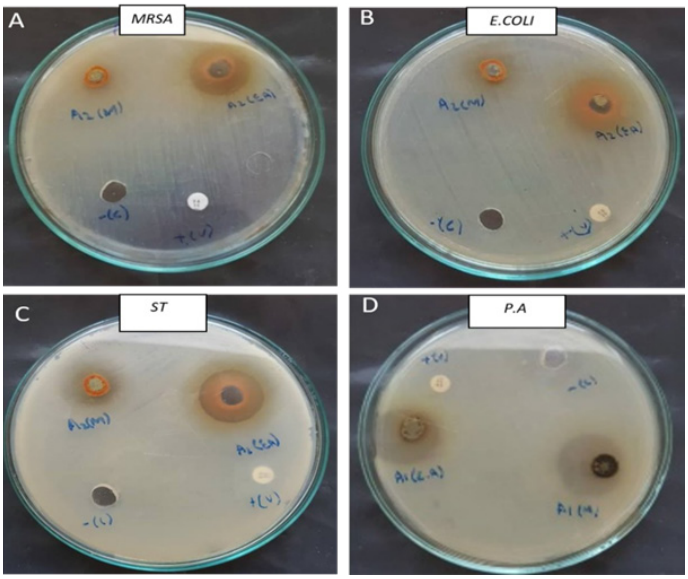

Figure 3: *Antibacterial activity of C. jacquemontii Plant extract (A) Methicillin resistant Staphylococcus aureus (MRSA), (B) E. coli (C) S. typhi, (D) P. auregenosa.*
Notes: (A2) M; Methanolic extract and (A1), Ethyl actetate extract, (C) Negetive control (10 % DMSO), Possitive control (+ive) Vancomycin.

Table 2: *C. jacquemontii (100 µg/ml) antibacterial activity displaying zone of inhibition (mm) and standard antibiotic Vancomycin (30 µg disc).*

Bacterial strains	Extract (ZOI)	Antibiotic (ZOI)
Pseudomonas aeruginosa(P.A)	20±1.167 mm	18 mm
Escherichia coli (E. coli)	18±2.306 mm	16 mm
Salmonella typhi (S.T)	17±2.306 mm	14 mm
Methicillin resistant staphylococcus aureus (MRSA)	15±2.306 mm	13 mm

Quantitative antioxidant activity of methanolic leaf extract of C. jacquemontii

The methanolic extract of *C. jacquemontii* were evaluated for quantitative antioxidant activity through DPPH scavenging assay. Extract's scavenging activities were compared with the activity of standard control, ascorbic acid. The extract showed a dose dependent color change, showing the amount of extract reacting with DPPH. At 150 µg/ml concentration of DPPH and 250 µg/ml concentration of extract dark black color was observed. These results demonstrated that the amount of extract will significantly affect the antioxidant activity represented by color changed in a dose dependent manner (Table 3).

An important aromatic plant with nutritional and medicinal benefits is *Corylus jacquemontii* (Decne). This plant is highly abundant in the District Kohistan Khyber Pakhtunkhwa, Pakistan and requires very little maintenance. In the current study, the phytochemical content, antibacterial, and antioxidant properties of the methanolic leaf extract of *C. jacquemontii* were investigated. Using a previously described approach, secondary metabolites such as alkaloids (3.60%), flavonoids (3.40%), and terpenoids (2.30%) were found in the methanolic fraction of *C. jacquemontii*, which demonstrated the highest level of phytochemical activity. *Gymnema sylvestre* were quantitively evaluated and showed the similar secondary metabolites (Kumar and Patra, 2017). The presence of different biologically active compounds such as alkaloids, flavonoids and terpenoids in plant extracts aided into plant different antimicrobial activities, hence use in traditional medicine (Chukwuka *et al.*, 2011; Khan *et al.*, 2021). Based on preliminary studies alkaloids and flavonoids are known to be biological response modifiers due to their role in modification of viruses, allergens, carcinogens. *Invitro* studies have shown their potential as an antimicrobial, anticancer, anti-allergic, anti-inflammatory (Spencer, 2008; Thawabteh *et al.*, 2019). Alkaloids are heterocyclic nitrogenous group of compounds having antimicrobial potential.

Table 3: *Antioxidant activity by qualtitative method for C. jacquemontii methanolic leaves extract.*

S. No	Conc of plant extract (µg/ml)	DPPH Conc (µg/ml)	Extract color	Control	Color of ascorbic acid
1	50	150	Dark Red	Ascorbic acid	Slightly white
2	100	150	Red	Ascorbic acid	Slightly white
3	150	150	Slightly red	Ascorbic acid	Slightly white
4	200	150	Slightly black	Ascorbic acid	Slightly white
5	250	150	Dark black	Ascorbic acid	Slightly white

The major mode of action of alkaloids is their intercalation with DNA (Setzer *et al.*, 2006). Flavonoids are considered natural antioxidants and antimicrobial agents by their ability to interact with bacterial cellular membrane, protein, and cell wall.

Similarly, the methanolic fraction of *C. jacquemontii* have good antibacterial activity against *P. aeruginosa,* and have a 20 mm zone of inhibition, as compared to the standard antibiotic, which have 18 mm zone of inhibition. The antibacterial potential tested with Broth dilution method also showed highest activity against *P. aeruginosa* with %MGI of 90±4.3 compared to vancomycin (61.26±6.31). Our results are closely related with a previous study (Ceylan *et al.*, 2013). They performed antibacterial activity of *C. colurna* leaf extract using methanol, dimethyl sulfoxide, distilled water, and petroleum ether. These extracts were also examined using the disc diffusion method to evaluate various Gram positive and Gram-negative strains. Another study also supported our results by evaluating the various extract of *Triphala* against different bacterial species and their antioxidant potential (Parveen *et al.*, 2018).

Similarly, the quantitative antioxidant activity of methanolic fraction of *C. jacquemontii* showed a dose dependent color changed, higher the amount of plant extract higher is the color change and hence higher the antioxidant activity. When DPPH was used at a concentration of 150 µg/ml and the extract was used at 250 µg/ml, a greater color shift was seen. This concentration showed a dark black color. This showed that extract amount has significant on antioxidant activity. Our results are supported by another study which evaluated the antioxidant activity on *C. colurna* by using the similar protocol (Riethmueller *et al.*, 2014). The presence of preferential quantity of different secondary metabolites in methanolic extract *C. jacquemontii* and its antibacterial potential as revealed in current study will further directed to emphasize on active compound purification.

Conclusions and Recommendations

C. jacquemontii is widely used as an anti-inflammatory, anticancer, antioxidant and cardio disorder protective remedy. Recent study showed that the methanolic leaves extract of the plant have promising phytochemical, antibacterial, and antioxidant activity. The main aim of current study is to focus on the medicinal potential of *C. jacquemontii* and the medicinal value of this plant suggest that future research should be conducted in a manner to keep in mind the properties of such wonder plant, while analyzing, isolating, characterizing the active compounds present in it.

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Novelty Statement

This is the first study reported on the biological evaluation on *Corylus jacquemontii* Decne., collected from District Kohistan Khyber Pakhtunkhwa, Pakistan.

Author's Contribution

Altaf ur Rehman, Muhammad Yahya, Muhammad Ajmal Khan: Conception and design.
Altaf Ur Rehman, Muhammad Yahya, Muhammad Ajmal Khan: Development of methodology.
Altaf Ur Rehman, Muhammad Ajmal Khan, Inam Ullah: Acquisition of the data.
Muhammad Ajmal Khan: Analysis and interpretation of data.
Altaf ur Rehman, Muhammad Ajmal Khan, Muhammad Yahya: Writing, review, and/or revision

of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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