



Antibacterial and Antifungal Properties of *Juglans regia* Hull

Dilshada Parveen*

Department of Botany, Central University of Kashmir, Ganderbal (J&K), India.

(Corresponding author: Dilshada Parveen*)

(Received: 14 January 2026; Revised: 16 March 2026; Accepted: 13 April 2026; Published online: 08 May 2026)

(Published by Research Trend)

DOI: <https://doi.org/10.65041/BiologicalForum.2026.18.5.6>

ABSTRACT: The emergence of multidrug-resistant pathogens and the ecological risks associated with synthetic agrochemicals have intensified the search for plant-derived antimicrobial agents. *Juglans regia* (walnut) hull, an abundant by-product of the nut industry, is rich in diverse phytochemicals including phenolic acids, flavonoids, naphthoquinones (notably juglone), and tannins, which are reported to exert antimicrobial effects. The present study systematically investigated the antibacterial and antifungal potential of *J. regia* hull extracts prepared using aqueous and methanolic solvents against a panel of clinically and agriculturally significant microorganisms. Phytochemical screening confirmed the presence of bioactive secondary metabolites with established antimicrobial relevance. The antimicrobial activity was evaluated through agar well diffusion and minimum inhibitory concentration (MIC) assays. The methanolic extract consistently demonstrated greater inhibitory zones compared to aqueous extracts, indicating higher solubility and bioavailability of phenolic compounds in organic solvents. Notably, methanolic extract exhibited strong antibacterial activity against *Klebsiella* (57.16 ± 2.64 mm zone of inhibition) and substantial antifungal effects against *Alternaria alternata*. Comparative analysis with standard antibiotic (ampicillin) revealed that *J. regia* hull extract possesses competitive inhibitory efficacy, underscoring its potential as a natural antimicrobial alternative. The findings highlight the therapeutic promise of *J. regia* hull as a sustainable and cost-effective source of novel antimicrobial agents. Beyond biomedical applications, its integration into food preservation, pharmaceutical formulations, and crop protection strategies could provide eco-friendly solutions to combat microbial resistance and reduce chemical pesticide reliance. Future research focusing on compound isolation, mechanism of action, and in vivo efficacy is warranted to advance the translational potential of this natural.

Keywords: *Juglans regia*, Hull, Antifungal, Antibacterial, Pathogens, Antimicrobial agents.

INTRODUCTION

Plants have been considered an essential source of medicine since ancient times, and are used in traditional systems of medicine, such as folk medicines (Sharma *et al.*, 2014). Plants have also been used as Ethno veterinary purpose. Plants are continually exposed to a wide spectrum of microbial organisms in their environment. Walnut are rich in proteins, fats, minerals, nutrients, and bioactive compounds such as phenolics, flavonoids, sterols, and polyphenols (Delaviz *et al.*, 2017). Green hull is a byproduct of the walnut (*Juglans regia* L.) and could be a potential source of phytochemicals with important bioactivities (Hosseini Hashemi *et al.*, 2025). Paulraj *et al.* (2025) also reported that *Juglans regia* root extract also shown antibacterial properties. Fungal and bacterial pathogens possess remarkable adaptive capabilities, allowing them to persist in diverse environmental conditions and across different ecological niches. Their ability to spread rapidly through air, water, soil, seeds, or vectors makes them difficult to contain,

especially in open agricultural systems. These pathogens can colonize virtually every part of a plant, including leaves, stems, roots, vascular tissues, and reproductive organs. Their impact is especially profound in both subsistence farming systems, where resource access is limited, and intensive commercial agriculture, where monocultures create ideal conditions for epidemics. The economic burden of microbial plant diseases is substantial. It is estimated that globally, crop yields are reduced by an average of 10–15% annually due to plant diseases, with losses often exceeding 20% in low-income or developing agricultural systems where disease management resources and diagnostic infrastructure are inadequate. According to the Food and Agriculture Organization (FAO), pests and diseases collectively account for approximately 25% of total crop losses worldwide. These losses not only affect farmers' incomes but also compromise national food security, market access, and international trade. Many strategies are in plea to tackle these issues of fungal and bacterial diseases however they have side

effects like for instance, the widespread use of chemical pesticides and fungicides can lead to the development of resistant strains of pathogens, disruption of beneficial soil microflora, environmental contamination, and adverse effects on human and animal health due to residual toxicity. However, it has been reported that the botanical has strong antifungal and antibacterial properties and same has been reported by many works. In this context we have used *Juglans regia* hull to a certain into antifungal and anti-bacterial property. *Juglans regia* hull extract is a promising natural antimicrobial agent, combining potent antioxidant activity with broad-spectrum antimicrobial efficacy. Its effectiveness depends on extraction solvent, cultivar, and ripeness stage. The hull's rich phytochemical profile, particularly phenolics and juglone, acts synergistically to inhibit both bacterial and fungal pathogens. These properties position the hull extract as a valuable candidate for applications in pharmaceuticals, food preservation, and alternative therapeutics—especially in the context of rising antimicrobial resistance.

Juglans regia is an evergreen tree commonly found in the sub-Himalayan regions, especially in Kashmir. It is rich in essential fatty acids, proteins, and lipids. The study aimed to ascertain the antibacterial and antifungal potential of the green hull extract of *J. regia*.

MATERIALS AND METHODS

Preparation of extract solution for antimicrobial testing.

125g of extract was weighed and dissolved in 100ml of Methanol to make the final concentration of 12.5mg/ml.

Bacterial suspension culture preparation.

Colonies from the selected pathogen were taken from pure culture plates and inoculation sterilized growth medium (Luria Bertanii broth) the suspension cultures were placed in incubator for incubation and shaking overnight at 37degree Celsius and 150rpm respectively

Preparation of plant extract. The hulls of *Juglans regia* were shade-dried, pulverized into fine powder (100–200 g), and subjected to extraction using methanol and aqueous solvents. For maceration, the powdered material was soaked in solvent (500–1000 mL) for 48–72 h with intermittent shaking, followed by filtration through Whatman filter paper. For Soxhlet extraction, powdered material was packed into a thimble and continuously extracted with solvent (500–1000 mL) for 2–3 h. The resulting extracts were filtered and stored.

To evaluate the antibacterial and antifungal properties of *Juglans regia* hull extract, selected bacterial and fungal strains were used as test organisms. The bacterial strains included both Gram-positive and Gram-negative species, while fungal strains were chosen based on their clinical and agricultural significance. Bacterial strains: e.g., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *CONS (Coagulase-negative staphylococci)*, *Klebsiella*, *bacillus subtills*, *Proteus mirabilis*. Fungal strains: e.g., *Alternaria alternate*,

The strains were obtained from SKUAST -K [biotechnology lab] and maintained on nutrient agar (for

bacteria) and potato dextrose agar (for fungi). Fresh subcultures were prepared before each experiment to ensure viability

Screening Methods for Antimicrobial Activity of Extract Solution

Well Diffusion methods (well variant):

In well diffusion method, solid agar medium (Meuller Hinton agar and MacConkey agar) was prepared and evenly inoculated it with the target microorganism/bacteria. Wells of size 6mm were created in the agar using a sterile borer (tip 200µl). The antimicrobial agent, plant extract, antibiotic (ampicillin) used as control was placed into these wells. The presence of a clear zone around the wells, known as the zone of inhibition, indicates plates were then incubated at 37°C overnight to allow the bacteria to grow. After incubation, antimicrobial activity. The diameter of these zones was measured to assess the effectiveness of the antimicrobial agent against the test microorganisms.

List of constituents for Well- Diffusion Methods

Constituents	Concentrations	volumes
MH-agar	38g in 1000ml (distilled water)	20ml
MacConkey agar	55.07g in 1000ml (distilled water)	20ml
Plant extract	12.5mg/ml	40µl
Ampicillin	12.5mg/ml	40µl
Methanol	Absolute	40µl

DISC DIFFUSION METHOD (Disc variant)

In disc diffusion method, bacteria were inoculated on a solid agar medium and with the help of a spreader, inoculum was uniformly spread across the surface. Sterile paper discs were impregnated with the antimicrobial plant extract, antibiotic, and Methanol separately and then placed on the agar surface. The plates were incubated to allow microbial growth at 37 °C overnight. After incubation, zones of inhibition around the discs, where the microbial growth has been prevented, were measured.

List of constituents for Disc diffusion method.

Constituents	Concentration	Volume
MH agar	38g in 100 ml (distilled water)	20ml
MacConkey agar	55.7g in 1000 ml (distilled water)	20ml
Plant extract	12.5mg/ml in DMSO	50µl
Gentamicin	12.5mg/ml	50µl
Ampicillin	Absolute	50µl

Time Killing Assay

The time-killing assay was performed to evaluate the antimicrobial activity of the plant extract and gentamicin using the MTT assay. Bacterial cultures were incubated in test tubes with the plant extract and gentamicin separately. At specific time intervals (0, 1, 2, 4, 8, and 24 hours), aliquots were taken and added to a 96-well microtiter plate. MTT solution was added to each well and incubated for 30 minutes at 37°C. After incubation, the formazan crystals formed were dissolved in DMSO, and the absorbance was measured at 565 nm using a microplate reader. The reduction in absorbance

compared to the control indicated the bactericidal effect of the treatments over time.

List of constituents for Time Killing Assay.

Test sample	Concentration	volume
Broth	40g/1000ml	5ml
Plant extract	0.5mg/ml	50µl
Gentamicin	0.5mg/ml	50µl
MTT	0.5mg	70µl

Colony Forming Assay

After performing the time-killing assay, 100 µl aliquots from each sample at 24 hours were taken and serially diluted in 1X PBS. Appropriate dilutions were plated on agar plates using the spread plate method to ensure isolated colonies. The plates were incubated at 37°C for 24 hours. After incubation, the plates were visualized to determine the number of viable bacteria. This allowed for the assessment of bacterial survival and the efficacy of the plant extract in reducing bacterial viability.

In Vitro Antifungal Assay

The antifungal activity of *Juglans regia* (walnut) hull extracts (prepared using methanol and water) was assessed against *Alternaria spp.* using the poisoned food technique described by Grover and Moore (1962). Potato Dextrose Agar (PDA) medium was supplemented with *J. regia* hull extracts at concentrations of 15 ppm, 30 ppm, and 60 ppm. Each treatment was conducted in triplicate.

After solidification of the medium, 6 mm mycelial discs from 8-day-old cultures of *Alternaria spp.* were placed at the center of each plate. Two sets of controls were maintained:

1. Pathogen control (PDA without any extract),
2. Solvent control (PDA containing only methanol or water, without extract).

The plates were incubated at 25°C for 7–10 days. Fungal growth was measured by recording colony diameter, and the percentage of mycelial growth inhibition was calculated using Vincent's formula (Vincent, 1947):

Inhibition (%) = $[(C - T) / C] \times 100$, where C = colony diameter in control, and T = colony diameter in treatment. The data obtained were statistically analyzed using one-way ANOVA and expressed as mean ± standard deviation (SD)

GC-MS Analysis of Phytoconstituents

Phytochemical profiling of the aqueous and methanolic extracts of *Juglans regia* hull was carried out using Gas Chromatography-Mass Spectrometry (GC-MS) at SKUAST-K Shalimar. The instrument was calibrated, and total ion chromatograms (TIC) were generated for both extracts. Identification of compounds was achieved by comparing retention times and mass spectra with entries in the Wiley Mass Spectral Library. Phytoconstituents such as flavonoids, phenolics, and organic acids were identified based on retention time, molecular ion peak, and fragmentation patterns (Sparkman *et al.*, 2011; Adams, 2007; Santos *et al.*, 2012).

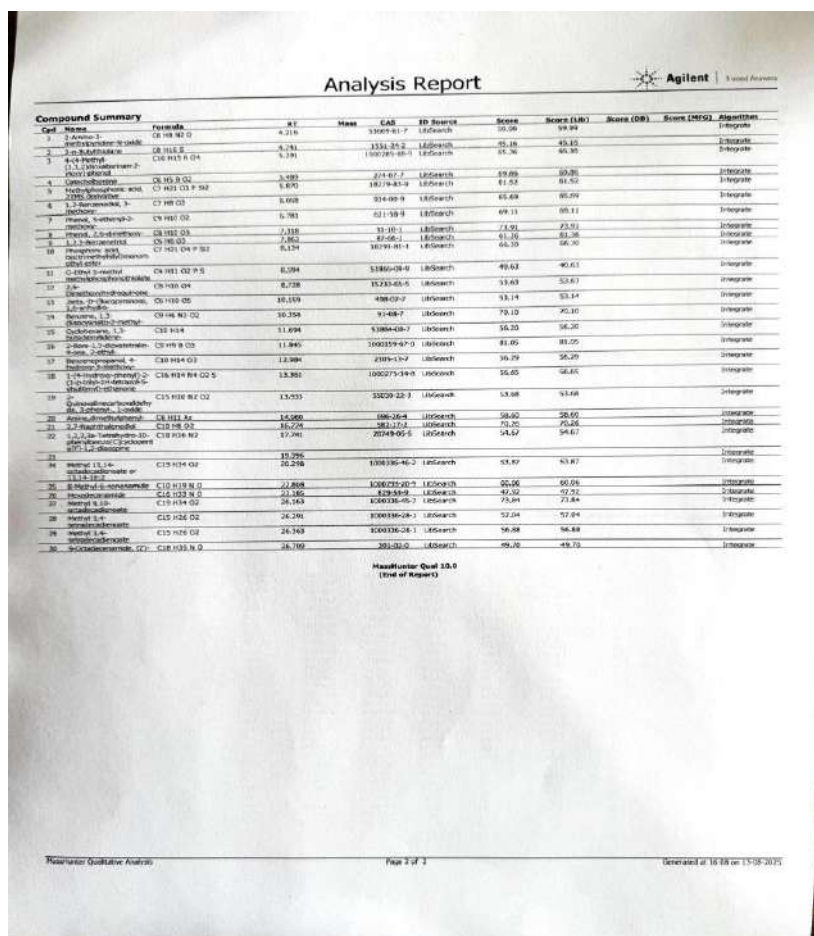
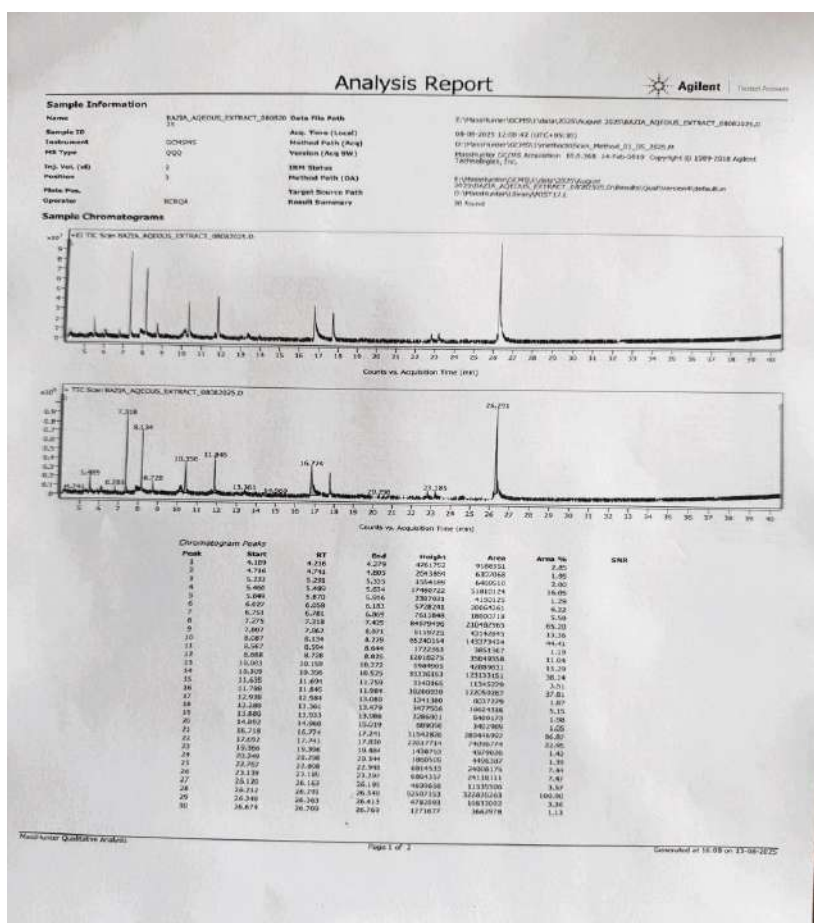
RESULTS

The preliminary analysis of *J. regia* hull showed presence of all basic compounds except Glycosides and proteins were absent in methanolic extract and proteins in aqueous extract as shown below (Table 1)

Table 1: Preliminary phytochemical screening of *J. regia* root hull extracts.

Phytoconstituents	Phytochemical Test	Extract of Hull of <i>J. Regia</i>	
		Methanol	Aqueous
Alkaloids	Dragendroff	++	+
	Wagners	++	+
Triterpenoids	Salkowski	++	+
Polysterols	Lindermann Burchards	++	++
Saponins	Alkaline Reagent	++	+
	Lead acetate	+	+
	Foam	+	+
Glycosides	Olive oil stain	+	+
	Anthraquinone	-	+
	Glycosides	-	+
Proteins	Phenolic compounds	++	+
	Biuret	+	-
Amino acids	Ninhydrin	-	+
	Xanthroproteic	+	+
Carbohydrates	Mollich's	+	+
	Barfoed's	+	+

The GC-MS analysis showed that both aqueous and methanolic extracts of *Juglans regia* hull showed good number of compounds. However, in methanolic extracts most of the compounds are showing Antifungal and Antibacterial properties (Fig. 1a-1d).



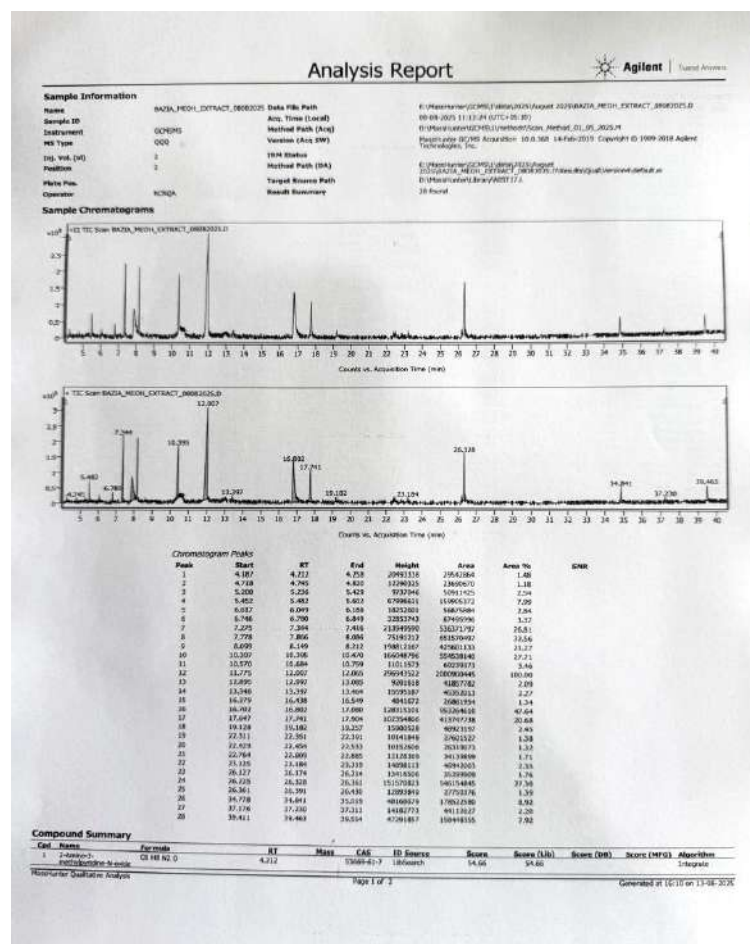


Fig. 1c. Chromatogram of Methanolic extract of *Juglans regia* Hull.

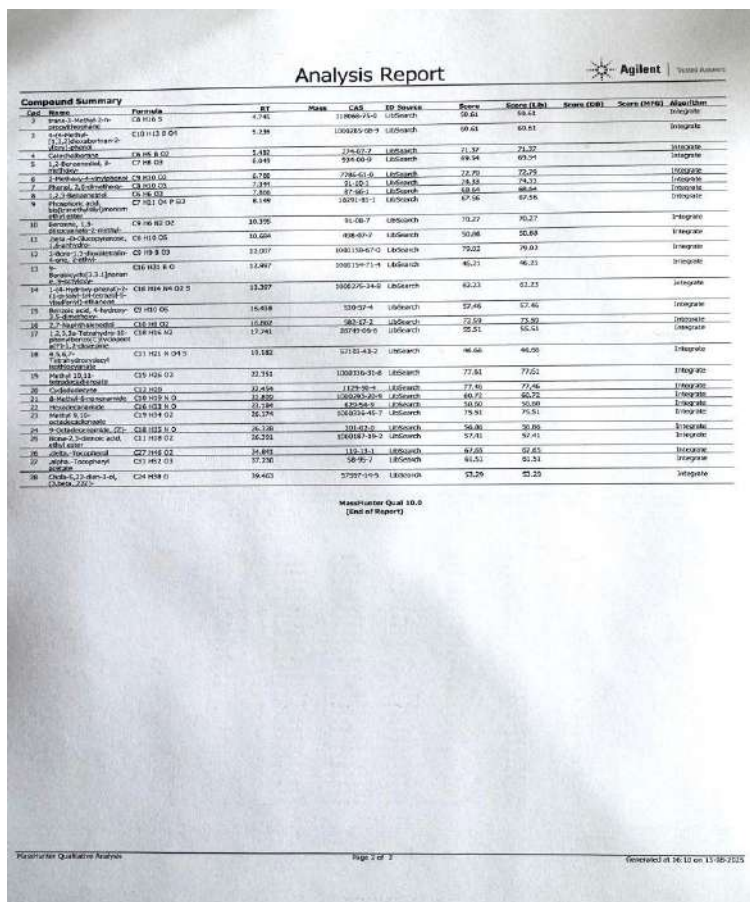


Fig. 1d. Phytochemical constituents of Methanolic extract of *Juglans regia* Hull.

Table 2: Inhibition zones measured against bacterial strains for methanolic and aqueous extract of *j regia* hull and standard ampicillin.

Bacteria	Control	Aqueous extract	Methanolic extract	Ampicillin
<i>P. aureus</i>	0	34.22±1.64 ^{hi}	40.10±1.62 ^{defgh}	36.45±1.21 ^{fghi}
<i>streptococcus</i>	0	38.32±2.42 ^{defghi}	49.24±2.64 ^{bc}	45.24±2.31 ^{cd}
CONS (<i>Coagulase-negative staphylococci</i>)	0	35.24±2.32 ^{ghi}	43.76±1.36 ^{cdef}	54.68±1.25 ^{ab}
<i>staphylococcus</i>	0	24.24±1.68 ^j	38.86±2.64 ^{defghi}	38.65±2.45 ^{defghi}
<i>Acinetobacter</i>	0	36.88±2.78 ^{efghi}	44.62±3.24 ^{cde}	32.24±2.14 ^{ij}
<i>Protus mirabelli's</i>	0	30.83±1.32 ^{def}	37.62±1.34 ^{defghi}	24.32±1.32 ^j
<i>Salmonella</i> spp	0	36.05±1.86 ^{fghi}	44.45±2.72 ^{cde}	36.24±2.32 ^{fghi}
<i>E. coli</i>	0	42.84±2.65 ^{cdefg}	42.34±2.18 ^{cdefg}	38.42±1.34 ^{defghi}
<i>salmonella</i>	0	10.14±0.85 ^k	47.92±2.64 ^{bc}	31.54±1.26 ⁱ
<i>Klebsiella</i>	0	42.84±2.14 ^{cdefg}	57.16±2.64 ^a	31.26±1.45 ⁱ

Table 3: Efficacy of *Juglans regia* hull extract on *Alternaria alternata*.

Concentration/treatment	Aqueous extract	Methanolic
15	21.16±1.24 ^a	54.76±3.14 ^a
30	26.82±1.62 ^b	63.84±3.24 ^b
60	31.64±1.32 ^d	72.76±2.14 ^c

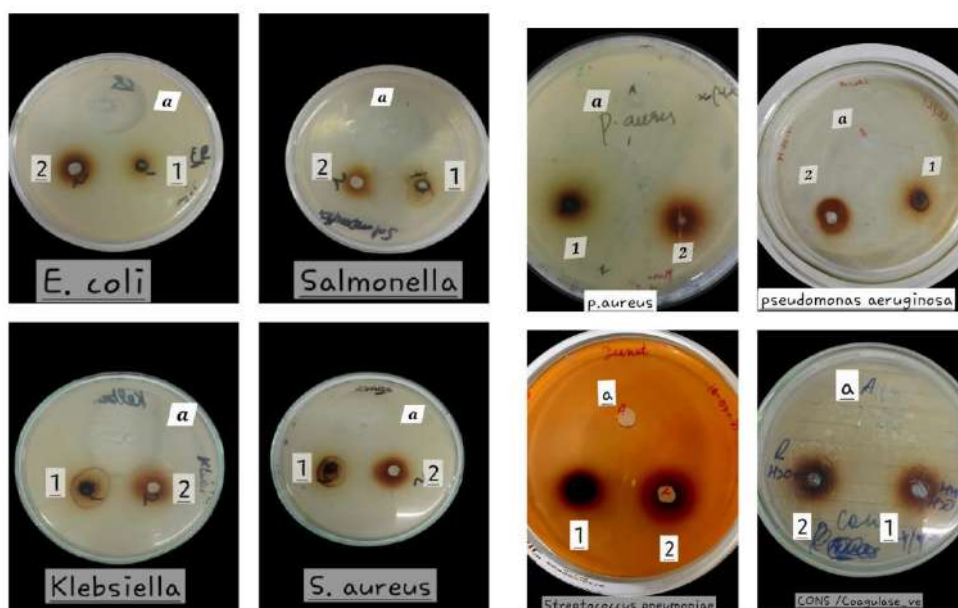
Aqueous extract –(*E- coli*)– The aqueous extract exhibited the greatest inhibitory effect, producing a zone of inhibition of 42.84±2.65 mm, followed by ampicillin (38.24 ± 1.34 mm) and the aqueous extract (42.34 ± 2.65 mm) The aqueous extract (***Klebsiella***) also exhibited the larger zone of inhibition, producing a zone of 4.84 ±2.14mm, followed by ampicillin (31.26±1.45) and the methanolic extract (57.16±2.64) (Table 2; Fig. 2).

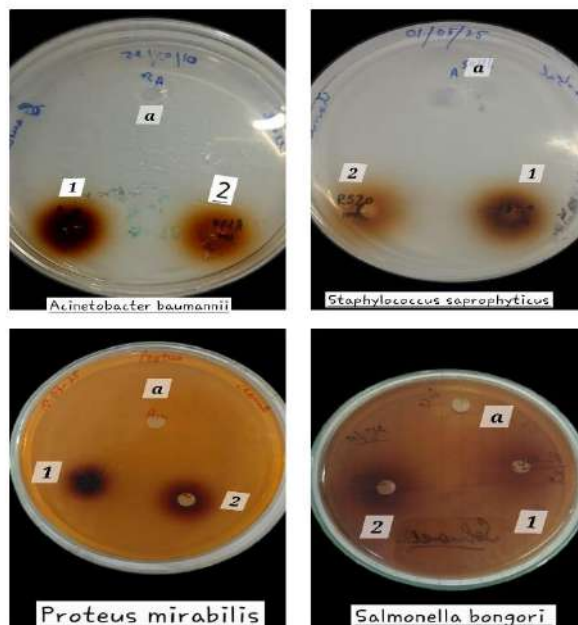
Methanolic extract of *klebsiella* inhibited larger bacterial growth with a zone of 57.16 ± 2.64) mm, while the aqueous extract produced 42.84 ± 2.14 mm and ampicillin inhibition of 31.26 ± 1.45 mm. The methanolic extract of ***Acinetobacter*** yielded 2nd larger zone of inhibition *i.e.* (44.62±1.36) mm, while aqueous

extract (36.88±2.78) and ampicillin (32.24±2.14) (Table 2; Fig. 2).

AMPICILLIN (antibiotic resistance) CONS has shown the larger inhibition zone *i.e.* (54.68± 1.25) mm. followed by methanolic extract (43.76 ±1.36) and the aqueous extract (35.24±2.32). (Table 2; Fig. 2). Overall, the methanolic extract exhibited superior antibacterial activity across most tested strains compared to the aqueous extract, indicating that methanol was more efficient in extracting antibacterial phytoconstituents from *J. regia* hull.

The aqueous and methanolic extracts showed good antifungal properties at higher concentration. However Methaloic extracts showed double the antifungal properties than the aqueous extracts (Table 3; Fig. 3).





(A= Ampicillin (control), (1 =Water.), (2= Methanol)
Fig. 2. Zone of inhibition against different pathogenic bacterial strains.



Fig. 3. Anti-fungal activity of Methanolic and Aqueous plant extracts against *Alternaria alternata*.

DISCUSSION

The management of fungal infections has become challenging due to antibacterial and antifungal drug resistance. Therefore, natural plant products are being explored for antifungal activity (Prasad *et al.*, 2016; Sytykiewicz *et al.*, 2015).

The outcomes of primary phytochemical screening of extracts of Hull of *J. regia* showed diversity in phytochemicals. The methanolic and aqueous extracts showed positive results for multiple phytoconstituents. Methanolic and aqueous extracts exhibited the existence of maximum constituent's viz., phytosterols, alkaloids, phenolics, triterpenoids, flavonoids, and saponins. Petroleum ether showed negative result for alkaloids, proteins, anthraquinone glycosides, and amino acids. These findings prompted to methanol and aqueous extracts (50 percent concentration) against bacterial strains. The findings showed prominent antimicrobial activity of *J. regia* Hull extracts. Three different concentrations of methanol and aqueous extracts (15, 30 and 60 percent) against *Alternata alternata*. However in both cases methanolic extracts showed significant results than aqueous extract. Same has been reported by Ara *et al.* (2023); Hosseini Hashemi *et al.* (2025) that methanolic extract showed significant results. The strong Antibacterial and Antifungal properties attributes to plants belonging to the Juglandaceae family possess diverse phytochemicals of therapeutic significance such as alkaloids, steroids (e.g., lupeol, betulinic acid, daucosterol, and β -sitosterol), tannins (e.g., casuarinin, stenophyllarin, glansrins A, B and C), flavonoids (e.g., catechin, epicatechin, gallo-catechin, procyanidin B2, epigallocatechin, and epicatechin gallate, phenolic acids (e.g., syringic, ferulic, caffeic, ellagic, gallic, p-coumaric, and sinapic acids, saponins, hydroxycinnamic acid derivatives, and terpenoids (Zhao *et al.*, 2014) (Ahmed, 2015; Paulraj *et al.*, 2025). The oil of *J. regia* consisted of juglone (A and B), juglone, 4-hydroxy-a-tetralone, α -pinene, β -pinene, germacrene-D, β -caryophyllene, limonene, camphene, sabinene, myrcene, and eugenol (Abdallah *et al.*, 2016; Devi *et al.*, 2011; Colaric *et al.*, 2005). The selected extracts displayed ample potential with regard to antimicrobial efficacy against microorganisms and may be utilized in mitigation of infectious pathologies of these microorganisms. Methanolic extract portrayed a good both Antibacterial and Antifungal activity.

J. regia should be explored to unearth a few bioactive natural compounds that might assist as lead compound and template for new antimicrobial pharmaceuticals. Thus, further work can be pursued and very much required for the isolation of pure phytochemicals and evaluation of their biological activities. The diverse spectrum of secondary metabolites identified—particularly phenolic compounds, alkaloids, saponins, and triterpenoids—suggests a multifaceted mode.

CONCLUSION

The findings from this study underscore the *Juglans regia* hull as a phytochemically rich matrix with substantial antimicrobial potential of action against both bacterial and fungal pathogens. The observed Anti-

bacterial and Anti-fungal activity showed that this waste product can be used to prepare bio-fungicide and bio-bactericide. Beyond its immediate therapeutic implications, the use of *J. regia* hull aligns with sustainable and low-waste bioprospecting practices, converting an agricultural byproduct into a high-value bioactive resource.

FUTURE SCOPE OF THE STUDY

Future work integrating phytochemical fractionation, mechanistic studies, and *in vivo* validation could pave the way for novel antimicrobial formulations capable of addressing multidrug-resistant microbial strains.

Acknowledgement. The author acknowledge the Central University of Kashmir and SKUAST-Kashmir for proving the facility for the completion of the work.

Conflict of interest. The author declare that there is no conflict of interest.

REFERENCES

- Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry: 978-1-932633-21-4, Carol Stream, Allured Publishing Co., Illinois, USA.
- Ahmed, H. (2015). Phenolics profile and content of walnut (*Juglans regia* L.) leaves in different cultivars grown in Iran. *Global Journal of Biochemistry and Biotechnology*, 3(2), 120-127.
- Abdallah, I. B., Baatour, O., Mechrgui, K., Herchi, W., Albouchi, A., Chalghoum, A. & Boukhchina, S. (2016). Essential oil composition of walnut tree (*Juglans regia* L.) leaves from Tunisia. *Journal of Essential Oil Research*, 28(6), 545–550.
- Ara, T., Shafi, S., Ghazwani, M., Mir, J. I., Shah, A. H., Qadri, R. A., Hakami, A. R., Khalid, M., Hani, U. & Wahab, S. (2023). In Vitro Potent Anticancer, Antifungal, and Antioxidant Efficacy of Walnut (*Juglans regia* L.) Genotypes. *Agronomy*, 13(5), 1232.
- Colaric, M., Veberic, R., Solar, A., Hudina, M. & Stampar, F. (2005). Phenolic acids, syringaldehyde, and juglone in fruits of different cultivars of *Juglans regia* L. *Journal of Agricultural and Food Chemistry*, 53(16), 6390–6396.
- Devi, T. N., Apraj, V., Bhagwat, A., Mallya, R., Sawant, L. & Pandita, N. (2011). Pharmacogenetic and phytochemical investigation of *Juglans regia* Linn bark. *Pharmacognosy Journal*, 3(25), 39-43.
- Delaviz, H., Mohammadi, J., Ghalamfarsa, G., Mohammadi, B. & Farhadi, N. A. (2017). Review Study on Phytochemistry and Pharmacology Applications of *Juglans regia* Plant. *Pharmacogn. Rev.*, 11, 145–152.
- Hosseini Hashemi, S. K., Baseri, H., Mehjabin, J. J., Alam, M. K., Hossin Ashrafi, S. K. & Lotfi, M. (2025). Phytochemical profile and antioxidant activity of *Juglans regia* L. green husk extracts from Tuyserkan region, Iran. *Bio Resources*, 20(3), 7265–7277.
- Prasad, R., Shah, A. H. & Rawal, M. K. (2016). Antifungals: Mechanism of Action and Drug Resistance. *Yeast Membr. Transp. Adv. Exp. Med. Biol.*, 892, 327–349.
- Paulraj, S., Ramakrishnan, H. & Javarai, P. (2025). Antibacterial Effect of *Juglans regia* Root Extract against Periodontal Pathogens: An *In Vitro* Study. *World J Dent.*, 16(4), 386-390.
- Santos, V. M. C. S., Pinto, M. A. S., Bizzo, H. & Deschamps, C. (2012). Seasonal variation of vegetative growth, essential oil yield and composition of menthol mint

- genotypes at southern Brazil *Bioscience Journal Uberlândia*, 28(5), pp. 790-798.
- Sharma, P., Gomathi, D., Ravikumar, G., Kalaiselvi, M. & Chandrasekaran, U. (2014). Phytochemical screening and high-performance thin layer chromatography fingerprinting analysis of green hull of *Juglans regia* (walnut). *Elect. Med. J.* 2, 235–239.
- Sparkman, D. O., Penton, Z. E. & Fulton, K. G. (2011). Gas chromatography and mass spectrometry: A practical guide (2nd Eds), 9780080920153, Elsevier Inc., Oxford, USA (2011).
- Sytykiewicz, H., Chrzanowski, G., Czerniewicz, P., Leszczyński, B., Sprawka, I., Krzyzanowski, R. & Matok, H. (2015). Antifungal activity of *Juglans regia* (L.) leaf extracts against candida albicans isolates. *Pol. J. Environ. Stud.* 24, 1339–1348.
- Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159, 239-241.

How to cite this article: Dilshada Parveen (2026). Antibacterial and Antifungal Properties of *Juglans regia* Hull. *Biological Forum*, 18(5): 36-44.