ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

Investigation of Anti-Microbial Activity of Juglans Regia L. against P. Aeruginosa and B. Subtilis

Aasif Manzoor Bhat[1]*; Mohd Altaf Khan[2]; Rashida Qureshi[3]; Mohd Yaseen[4]

[1;4]Center of Research for Development University of Kashmir, Srinagar, India
[2]Department of Environmental sciences, Lovely Professional University, Punjab, India
[3]Department of Chemistry, Saifia Science College, Madhya Pradesh, India

Corresponding Author: Aasif Manzoor Bhat[1]*

Publication Date: 2025/06/24

Abstract: Antibacterial activities of methanolic extract and ethyl acetate extract of *Juglans regia* . fruit husk cultivated in Kashmir were probed. Hot extraction process with methanol and ethyl acetate was carried out by employing Soxhlet apparatus method . The antimicrobial activity was calculated employing agar diffusion method. The extracts of fruit husk manifested antimicrobial activity against Pseudomonas aeruginosa as well as Bacillus subtilis along with minimum inhibitory concentration (MIC) of 100 µg/mL for Juglans regia (EA) extract against Pseudomonas aeruginosa,50 µg/mL for Juglans regia (MeOH) extract against Pseudomonas aeruginosa, 100 µg/mL for Juglans regia (EA) extract against Bacillus subtilis and 50 µg/mL for Juglans regia (MeOH) extract against Bacillus subtilis. The outcome established that the methanolic extract of Juglans regia fruit husk display antibacterial property against Pseudomonas aeruginosa and Bacillus subtilis.

Keywords: *Juglans Regia Husk; Methanol Based Extract; Microbicidal Activity; Minimum Inhibitory Concentration.*

How to Cite: Aasif Manzoor Bhat; Mohd Altaf Khan; Rashida Qureshi; Mohd Yaseen (2025) Investigation of Anti-Microbial Activity of Juglans Regia L. against P. Aeruginosa and B. Subtilis. *International Journal of Innovative Science and Research Technology*, 10(6), 1752-1759. https://doi.org/10.38124/ijisrt/25jun113

I. INTRODUCTION

Antimicrobial drugs are vital for the remedy of bacterial sickness in human beings and animals. They have transformed medical treatment operations globally. For example, penicillin taken down death associated with pneumococcal pneumonia from 25- 45% to 6% and death arising out of pneumococcal bacteremia from 60- 75% to 18%. However, bacterial defiance to antimicrobials swiftly garnish a considerable clinical challenge frightening the developments of the decades before and constituting a notable threat to public health.

The necessity for new antimicrobials has been increased spectacular. Plants are regarded as one of the most auspicious sources for new antimicrobial discovery. There are more than 1350 plants having explicate antimicrobial properties and above 30,000 antimicrobial compounds are being segregated out of plants.

The large scale, inappropriate, ill-suited, and spasmodic usage of antibiotics have culminated in the disclosure of resistance to antibiotics, making multiple presently available prescription drugs ineffectual. Phytomedicine was and is yet one of the leading beneficences in the remedy of distinct maladies. Multitude of plants were accounted as notable ingredient in natural medicine. An instance of one out of the significant and therapeutically functional plants is Juglans regia, which is related to Juglandaceae clan. It's routinely referred as walnut and is spread all-over the globe. The root, bark and seed of Juglans regia are harnessed to alleviate a range of medical conditions. Antimicrobial features of the Juglans were proclaimed formerly for distinct parts of the plant. In the ongoing investigation methanolic and ethyl acetate extract of Juglans regia fruit husk have been scrutinized for its antimicrobial activity which is ascribed to its multiple chemical components.

II. MATERIALS AND METHODS

- *Fruit Husk Collection*

The sample plant material of fruit husk of Juglans regia was gathered from autochthonous region in Kashmir in the month of August -2021 and authenticated by Centre for Biodiversity, University of Kashmir and documented (voucher specimen ID 4312-KASH). The fruit husk were air parched under the shade at moderate temperature to make powder after pulverizing.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

➢ *Preparation of Extract*

A hot continuous extraction procedure was executed coinciding to the standard procedure reported in literature. The grated shriveled fruit husk of the sample plant (137.97g) was put on the inside of a paper timber where the sample plant material was impregnated at normal temperature and pressure all night containing 250 ml of methanol and 250 ml of ethyl acetate separately. Next the sample plant material was put to Soxhlet extrication for 10 h at a highest temperature of 55 ºC. The raw extracts were vaporised to acquire the dehydrated extract.

➢ *Microorganisms*

The antimicrobial activity of methanolic extract and ethyl acetate extract acquired from Walnut was tested in contrast to Pseudomonas aeruginosa (MTCC 8076) a gram-negative bacteria and Bacillus subtilis (MTCC 736) gram-positive bacteria . The bacteria were incipiently revitalized from reference cultures, and also kept-up on Mueller Hinton broth (MHB) at 37 ˚C for 20 hours.

III. ANTIBACTERIAL ACTIVITY

➢ *Well Diffusion Assay*

- *Nutrient Media Preparation*

28 g of agar nutrient media was dispersed in 1000 ml of purified water. Hydrogen ion concentration of media was inspected before disinfection. In autoclave media was disinfected at 120 ^{0}C at 15 lbs pressure for 20 minutes. Media was permitted to cool after decontamination but not let to solidify. In the laminar air flow nutrient media was flushed into plates and set in as late as the agar gets solidified.

- *Well Diffusion Assay*

Culture of microbial strains were roll out on the agar nutrient media. Next 1ml of trial sample (Juglans regia, and methanolic and ethyl acetate extract) was withdrawn striaght from the reference solution. Next 10 mg of stock (Amoxycillin and Ofloxacin) was withdrawn with 1ml purified water to prepare 10mg/10ml of solution. Then, inseminate of Bacillus subtilis (MTCC 736) a gram positive bacteria and Pseudomonas aeruginosa (MTCC 8076) a gram negative bacteria was make ready; experimental organisms were inculcated in 10ml of Nutrient bouillon. The bacterial culture was regularised to 108 CFU/ml of bacteria and retained within the shaker. Then, inoculum of the 50µl was taken out of the broth (holding 108 CFU/ml) was withdrawn with micropipette and then transmitted to new and disinfected frozen agar plate media. (Mohammadi-Sichani et al., 2012). The agar plate was infused by straightened out the inoculum with a disinfected diffuser, above the whole sterile agar stretch. The 6 mm four wells were cut in the culture media with the aid of disinfected cork-borer. Every single well was loaded with varying concentrations (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) of plant sample and one more plate well was loaded with 50µl of established drug separately. It was permitted to scatter for about 40 minutes at normal temperature and cultivated for 20-25 hours at 37 ^{0}C. Following preparation, plates were noticed for the development of a clean zone on every side of the well that correlates to the antimicrobic property of examined compounds. The zone of inhibition (ZOI) was watched and calculated in mm. Zones are calculated to a near by millimeter employing a ruler, which was kept on the rear end of the upend Petri plate. The Petri plate were kept a few inches over-head a black, anti-reflecting back-drop. The perimeter of the zone of absolute obstruction (as judged by bare eye) were calculated, as well as the diameter of the well (Manandhar et al., 2019).

➢ *Minimum Inhibitory Concentration*

- *Preparation of Dilution of Sample*

Two-fold sucessive dilution of plant extracts (Juglans regia, methanolic and ethyl acetate extract) were make ready in disinfected test tubes starting with 1:1 undiluted in addition to 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

➢ *MIC Procedure*

- *Inoculum Preparation*

The one gram positive (Bacillus subtilis (MTCC 736) bacterial suspensions and one gram negative (Pseudomonas aeruginosa (MTCC 8076) was make ready by regulating the turbidity around 0.5 McFarland. 0.5 McFarland suspensions was subsequently diluted and brought out at 1:10 with decontaminated nutrient broth to procure an108 CFU/ml of inoculum. (Yousef and Danial, 2012)

- *Protocol*

Calculations of the MICs of examination sample (extracts) against the strains above were set on by broth dilution employing stepwise 1:2 dilutions in the nutrient broth medium.

The Minimum Inhibitory Concentration was brought out in ten hatches of uninfected test apparatus tubes. Shortly, the parent infusion of test sample (Juglans regia, methanolic and ethyl acetate extract) was make ready in ethyl acetate and methanol to make certain total dissolution in a concentration of 1 mg/ml. A sum of Nutrient Broth of 3ml was distributed in 1st test tube to a test tube 10. Trial sample (extracts) (400 µl) was add up to the test tube one. The infusion was stepwise diluted through test tube 1 to test tube 10 while 400 µl was junked from test tube 10. Bacterial suspension of 200 µl was add on to all dilutions extends from test tube 1 to test tube 10. Through out the night bacterial inoculum (200 µl) was distributed in test tube 11 and 3 ml of aseptic broth was put on to be employed as standard positive control while 3 ml of aseptic Nutrient Broth in 12th test tube which served as negative control . The test tubes were cultivated at 37 ^{0}C for 24 h. Following incubation, absorbance of every single test tube was gauged employing 640nm wavelength UV-Visible spectrophotometer (Systronics 2202). The afore-mentioned course of action was executed for every microbial strain. The sample and standard concentration which hindered bacterial growth up to 50 percent was checked for all microorganisms (Parvekar et al., 2020).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

IV. RESULTS AND DISCUSSION

➢ *Antimicrobial Activity of Juglans Regia Extract*

Table 1 Antimicrobial Activity of Juglans Regia (MeOH) Extract against B. Subtilis Based on Figure 1,2,3

Concentration (µg/ml)	Plate 1 Zone of inhibition (mm)	Plate 2 Zone of inhibition (mm)	Plate 3 Zone of inhibition (mm)	Mean±SD (mm)
100	7	7	8	7.33±0.577
75	0	0	0	0±0
50	0	0	0	0±0
25	0	0	0	0±0

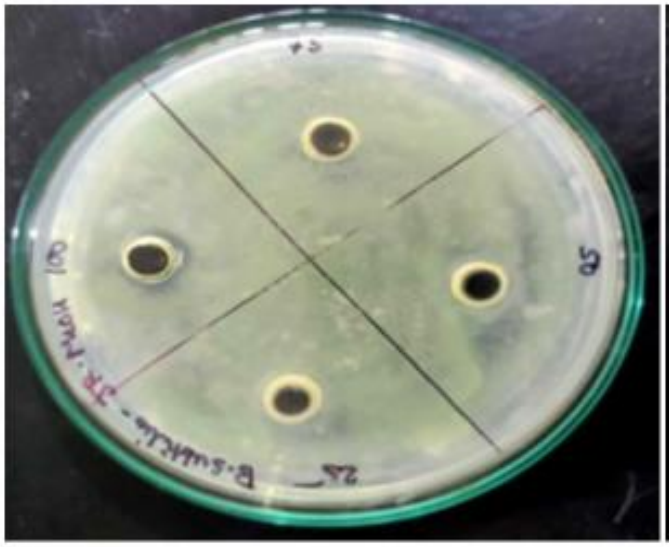

Figure 1

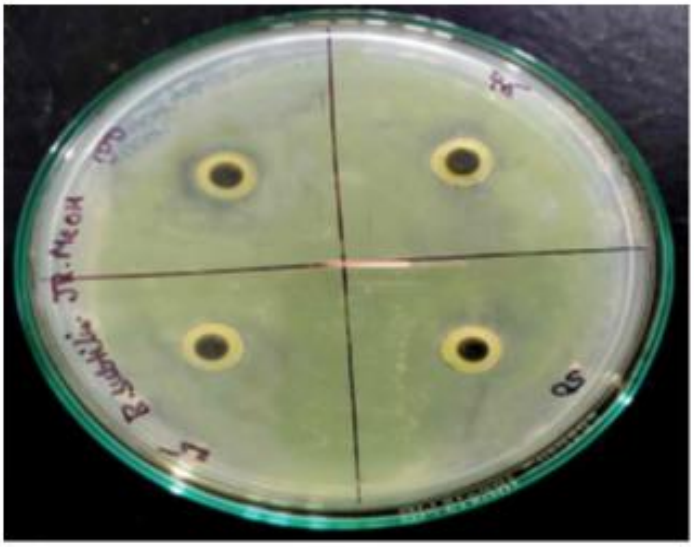

Figure 2

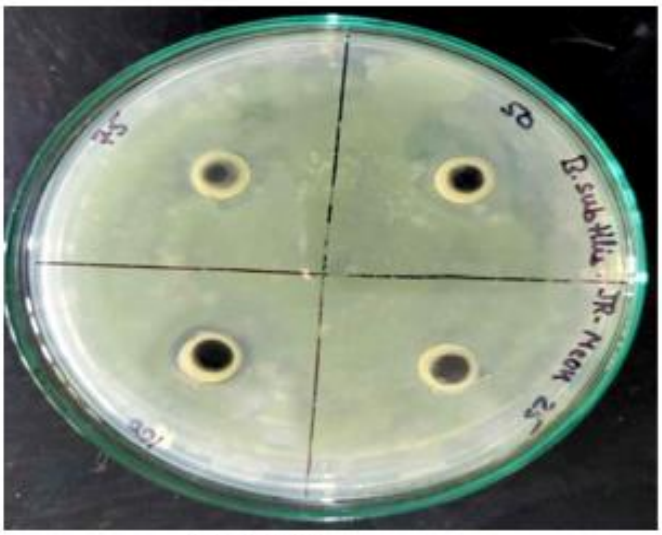

Figure 3

Fig 1,2,3 Represents Activity of Juglans Regia Methanol Extract against B. Subtilis

Table 2 Antimicrobial Activity of Juglans Regia (MeOH) Extract against P. Aeroginosa Based on fig. 4,5,6

Concentration (µg/ml)	Plate 1 Zone of inhibition (mm)	Plate 2 Zone of inhibition (mm)	Plate 3 Zone of inhibition (mm)	Mean±SD (mm)
100	8	9	7	8±1
75	0	0	0	0±0
50	0	0	0	0±0
25	0	0	0	0±0

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

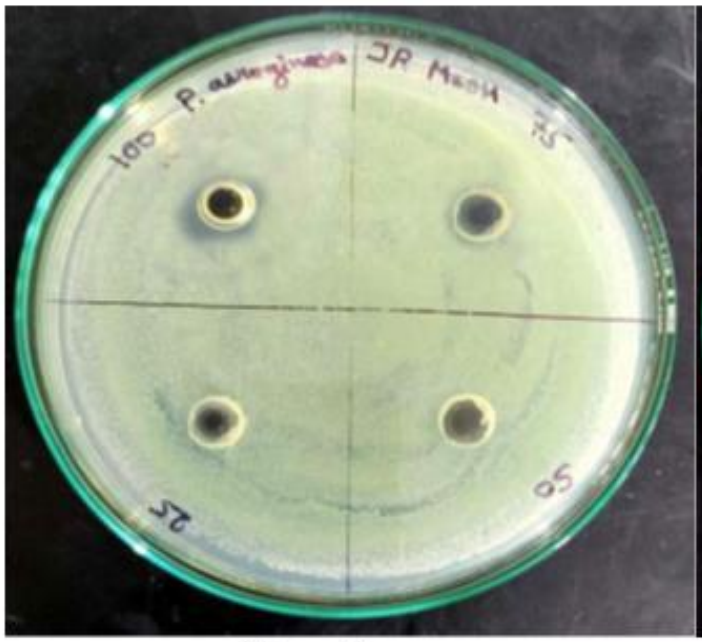

Figure 4

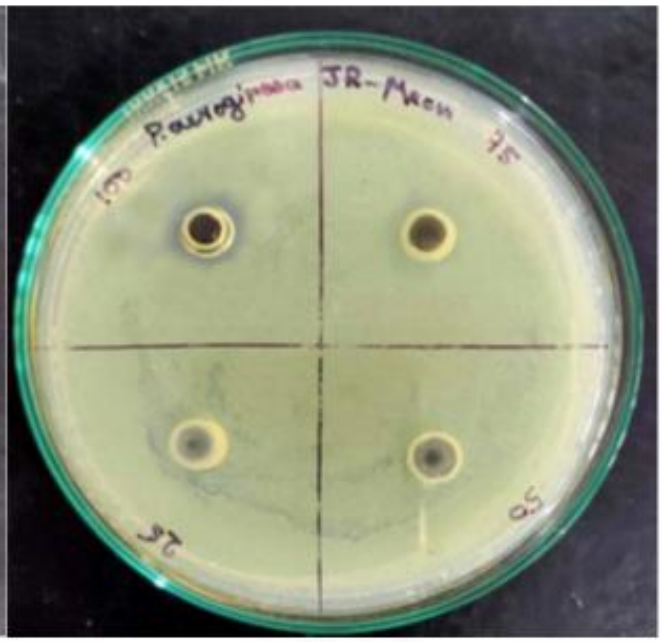

Figure 5

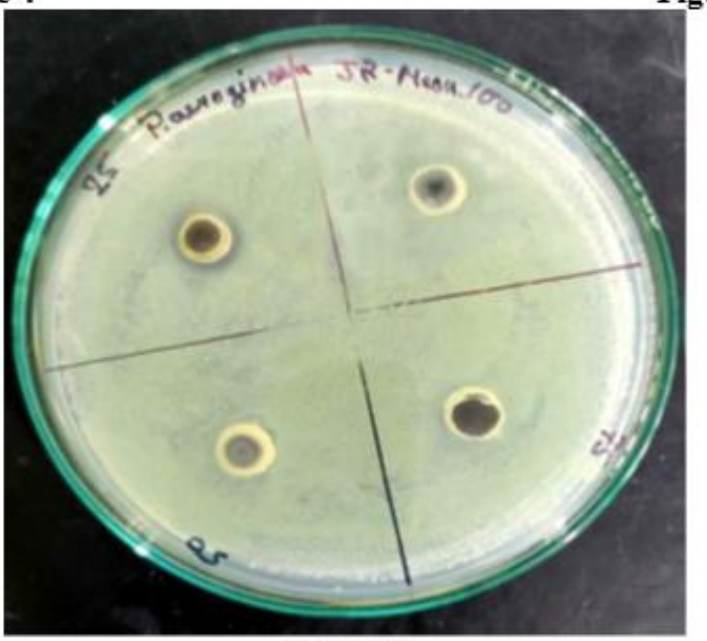

Figure 6

Fig 4,5,6 Represents Activity of Juglans Regia Methanol Extract against P. Aeroginosa

Table 3 Antimicrobial Activity of Juglans Regia (E.A.) Extract against P. Aeroginosa Based on Figure 7,8,9

Concentration (µg/ml)	Plate 1 Zone of inhibition (mm)	Plate 2 Zone of inhibition (mm)	Plate 3 Zone of inhibition (mm)	Mean±SD
100	7	7	7	7±0
75	0	0	0	0±0
50	0	0	0	0±0
25	0	0	0	0±0

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

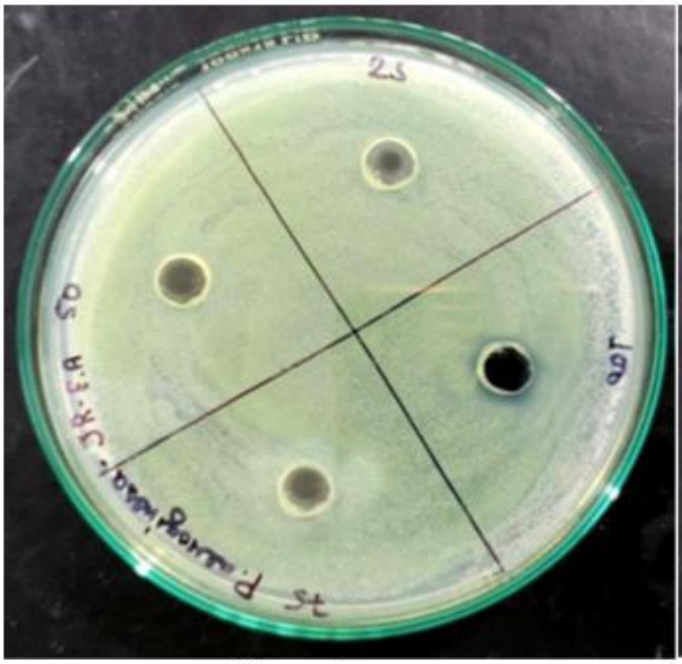

Figure 7

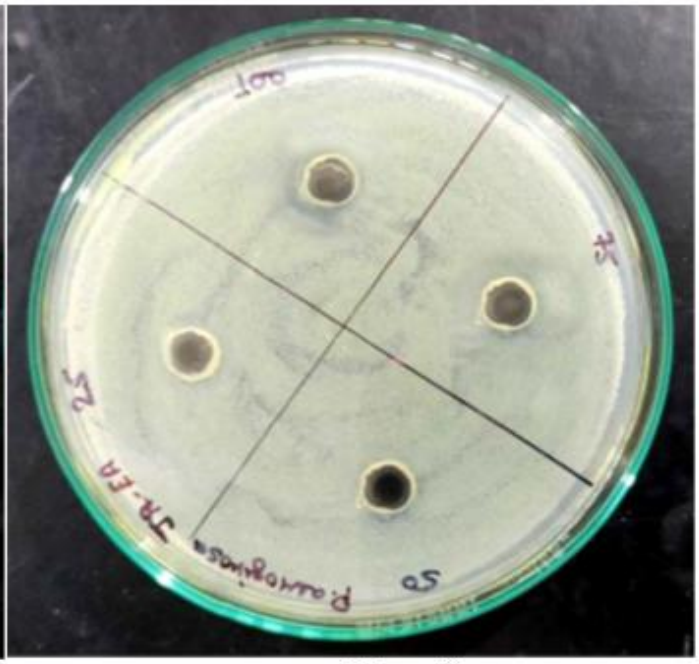

Figure 8

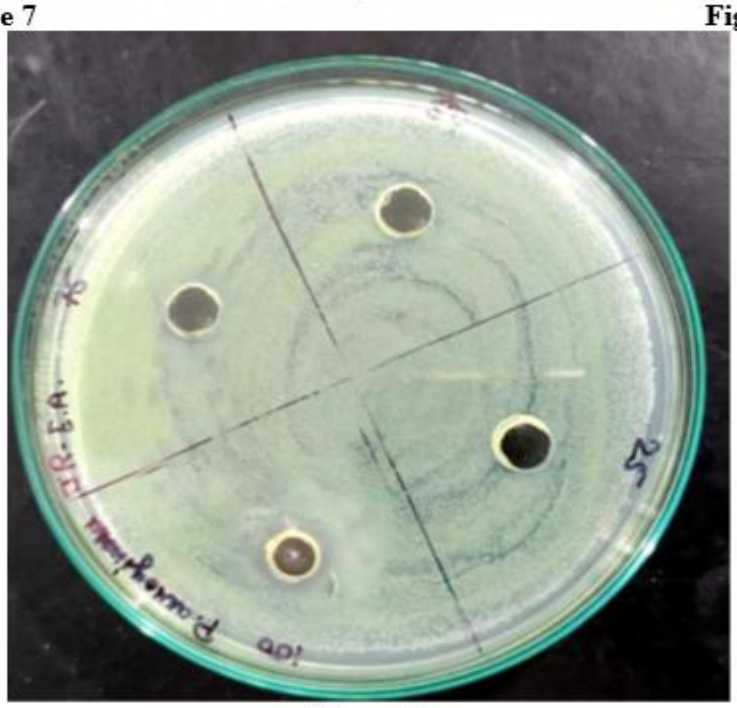

Figure 9

Fig 7,8,9 Represents Activity of Juglans Regia Ethyl Acetate Extract against P. Aeroginosa

Table 4 Antimicrobial Activity of Juglans Regia (E.A.) Extract against B. Subtilis Based on Figure 10,11,12

Concentration (µg/ml)	Plate 1 Zone of inhibition (mm)	Plate 2 Zone of inhibition (mm)	Plate 3 Zone of inhibition (mm)	Mean±SD
100	8	7	7	7.33±0.577
75	0	0	0	0±0
50	0	0	0	0±0
25	0	0	0	0±0

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

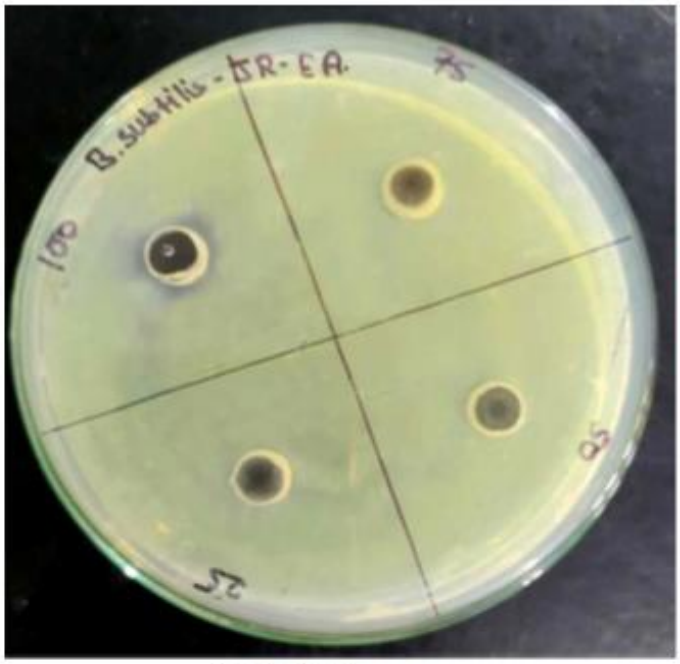

Figure 10

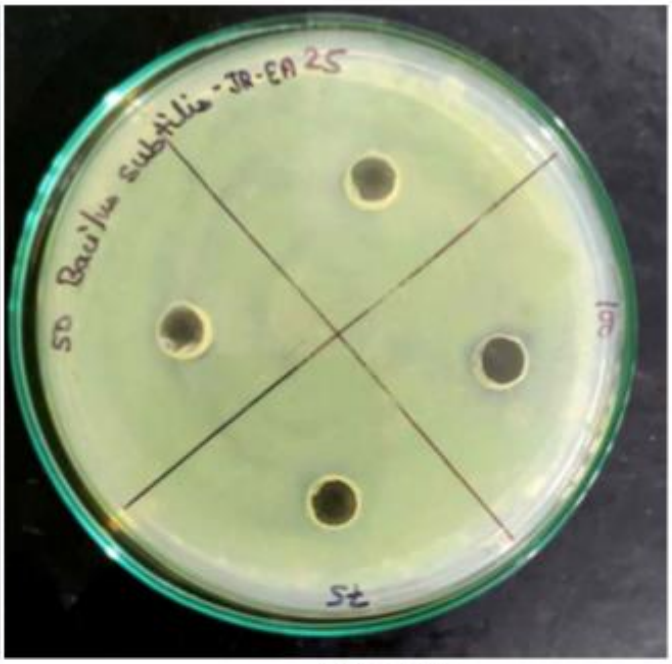

Figure 11

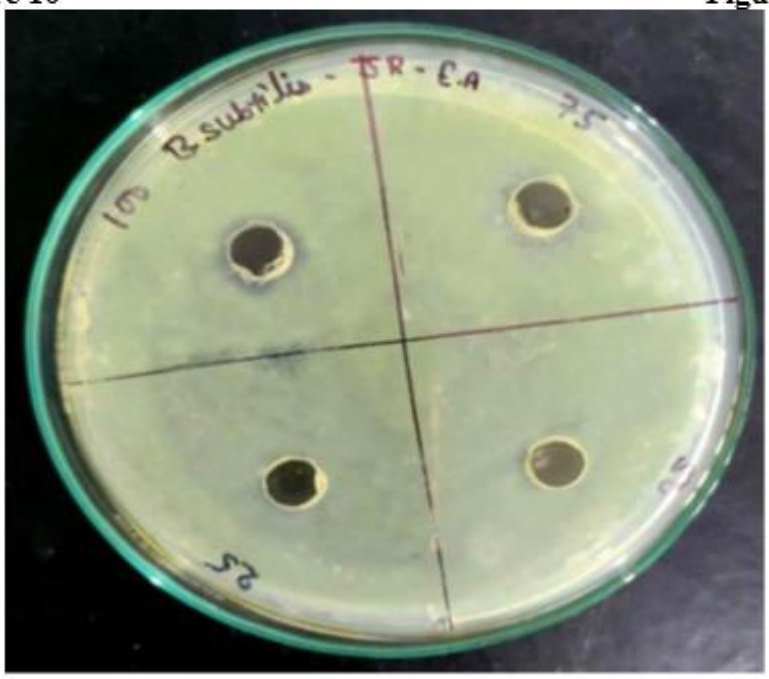

Figure 12

Fig 10,11,12 Represents Activity of Juglans Regia Ethyl Acetate Extract against B. Subtilis

- *Antimicrobial Activity Results of Standard against Gram Positive and Gram-Negative Bacteria*

Table 5 Antimicrobial Activity of Standard against Gram Positive and Gram-Negative Bacteria Based on Figure 13, 14

Microorganisim	**Zone of inhibition (mm)**
Gram positive bacteria (*B. subtilis*)	30.2 mm
Gram negative bacteria (*P. aeruginosa*)	32 mm

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

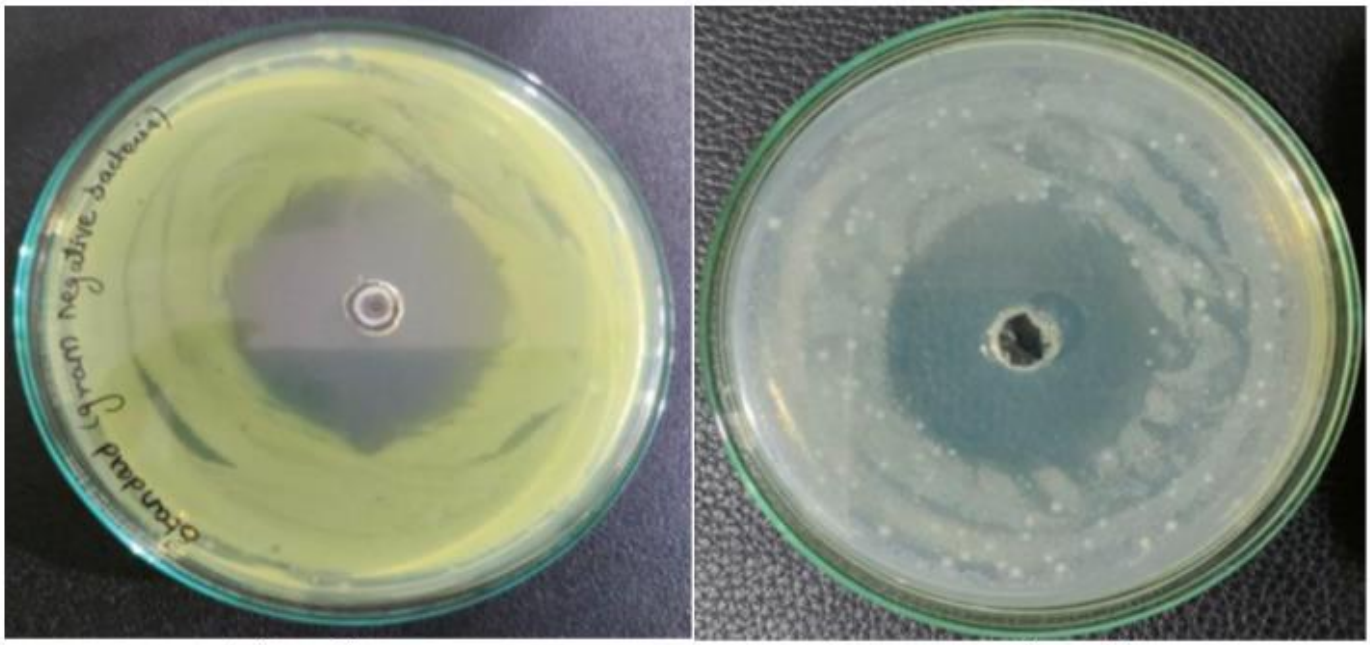

Fig 13 Antimicrobial Activity of Standard (Amoxycillin) against Gram Negative
Fig 14 Antimicrobial Activity of Standard (Ofloxacin) against Gram Positive

➢ *Results of Minimum Inhibitory Concentration*

MIC is demonstrated as the minimal Concentration of sample, which inhibited growth determined by lack of turbidity in the well.

➢ *MIC of Standard Drug*

Table 6 Results of MIC of Standard against Bacteria

S. No	Sample name	Bacteria name	Minimum Inhibitory Concentration (µg/ml)
1.	Amoxycillin	B. subtilis	0.39 µg/ml
2.	Ofloxacin	*P. aeruginosa*	0.19 µg/ml

➢ *Minimum Inhibitory Concentration (MIC) for Plants Tested*

Table 7 Results of Extract against Bacteria

S. No	Extract name	Bacteria name	Minimum Inhibitory Concentration (µg/ml)
1.	*Juglans regia* (EA) extract	*P. aeruginosa*	100 µg/ml
2.	*Juglans regia* (MeOH) extract	*P. aeruginosa*	50 µg/ml
3.	*Juglans regia* (EA) extract	*B. subtilis*	100 µg/ml
4.	*Juglans regia* (MeOH) extract	*B. subtilis*	50 µg/ml

V. CONCLUSION

In compendium, the methanolic extract and ethyl acetate extract of J.regia fruit husk was investigated for its antimicrobic properties. The methanolic extract was functional against Gram-positive and Gram-negative bacteria, unmasking antimicrobial properties. Addtionally, the extract generated anti-oxidant response. The revelations of this study indicate possible use of J.regia fruit husk extract as antimicrobial. Inspite of that, clinical studies are also needed in order to probe other viable pharmacological activities, well being and effectiveness. The current study justified the proclaimed uses of fruit husk in the customary system of medicine to cure numerous infectious disease induced by the microbes. However, additional studies are required to better evaluate the potential potency of the crude extracts as the microbicidal. Additional investigations are imperative to evaluate antimycotic, antiviral, and parasiticide activity. In addition, other segments of the plants need to be considered to analyse the contrived plant extracts as a prospective antimicrobial agent.

ACKNOWLEDGMENT

The authors are grateful to the Center of Research for Development University of Kashmir, Srinagar for substantial support.

➢ *Conflict of Interest*

The authors views are impartial and unbiased.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun113

REFERENCES

[1]. Sharma P, Ravikumar G, Kalaiselvi M, Gomathi D, Uma C. In vitro antibacterial and free radical scavenging activity of green hull of Juglans regia. Journal of pharmaceutical analysis. 2013 Aug 1;3(4):298-302.

[2]. Firenzuoli, F.; Gori, L. Evid Based Complement Alternat Med. 2007, 4, 37-40.

[3]. Girzu M, Carnat A, Privat AM, Fialip J, Carnat AP, Lamaison JL. Sedative effect of walnut leaf extract and juglone, an isolated constituent. Pharmaceutical biology. 1998 Jan 1;36(4):280-6

[4]. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010 Dec 10;2(12):1231-46

[5]. Shojaii, A.; Dabaghian, F. H.; Goushegir, A.; Fard, M. A. Acta Med Iran. 2011, 49, 637-42. .

[6]. Hu Q, Liu J, Li J, Liu H, Dong N, Geng YY, et al. Phenolic composition and nutritional attributes of diaphragma juglandis fructus and shell of walnut (Juglans regia L.). Food Sci Biotechnol 2020;29:187-9

[7]. Jahanban-Esfahlan A, Ostadrahimi A, Tabibiazar M, Amarowicz R. A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (Juglans regia L.) fruit and tree. Molecules 2019;24:2133.

[8]. Bahmani, M.; Rafieian-Kopaei, M.; Hassanzadazar, H.; Saki, K.; Karamati, S. A.; Delfan, B. Asian Pacific journal of tropical medicine. 2014, 7, S29-S33.

[9]. Li H, Horke S, Förstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 2013;34:313-9.

[10]. Kavak DD, Altiok E, Bayraktar O, Ülkü S. Pistacia terebinthus extract: as a potential antioxidant, antimicrobial and possible β-glucuronidase inhibitor. J Mol Catal B Enzym 2010;64:167-71.

[11]. Acquaviva R, D'Angeli F, Malfa GA, Ronsisvalle S, Garozzo A, Stivala A, et al. Antibacterial and anti-biofilm activities of walnut pellicle extract (Juglans regia L.) against coagulase-negative staphylococci. Nat Prod Res 2021;35:2076-81.

[12]. Chopra, R.; Nayar, S.; Chopra, I. New Delhi. 1986, 2-79.

[13]. Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radic Biol Med 1999;27:1173-81.

[14]. Kusano C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Cell Mol Biol 2008;7:1- 15.

[15]. Chibane LB, Degraeve P, Ferhout H, Bouajila J, Oulahal N. Plant antimicrobial polyphenols as potential natural food preservatives. J Sci Food Agric 2019;99:1457-74.