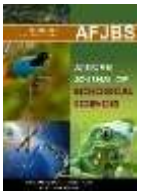
https://doi.org/10.48047/AFJBS.6.13.2024. 1787-1801

African Journal of Biological Sciences



ANTIMICROBIAL EFFECTS OF *JUGLANS REGIA* LINN., LEAF EXTRACT AGAINST SELECTED PATHOGENIC BACTERIA AND FUNGAL SPECIES

Noel Vinay Thomas[1*], Sushruta Koppula[2], Nabaz Hama Rashid[1], A Salomy Monica Diyya[3], Dlzar Dlshad Ghafour[1, 4], Hozan Fadeel Ghareeb[1], Naz Kamaran Ahmed[1], and Kozhir Karim Abdullah[1]

[1] College of Science, Department of Medical Laboratory Science, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq.

[2] College of Biomedical and Health Sciences, Konkuk University, Chungju 27478, Republic of Korea.

[3] College of Medicine, Department of Pharmacy, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq

[4] College of Science, Department of Chemistry, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq

*Corresponding Author: noel.thomas@komar.edu.iq Tel: +964 750 110 3131 - EXTN: 158

Article History
Volume 6, Issue 13, 2024
Received: 18June 2024
Accepted: 02July 2024
doi:*10.48047/AFJBS.6.13.2024.* 1787-1801

Abstract

Juglans regia, commonly known as English or Persian walnut is a deciduous tree with high nutritional, medicinal as well as economical value. To our knowledge, the medicinal importance of *J. regia* as anti-inflammatory, antibacterial and anti-fungal is not fully exploited. Therefore, the current study focused on the antimicrobial potential of the *J. regia's* leaf extracts against several bacteria and fungi such as *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus (MRSA), Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* (multi drug resistant), *Klebsiella pneumonia*, *Klebsiella pneumonia* (multi drug resistant), *Escherichia coli* and *Aspergillus Flavus, Fusarium, Penicillium.* We evaluated the antibacterial and anti-fungal effects of the leaf extracts using soxhlet extraction in ethanol, methanol and water as solvents and water bath extraction, ethanol, and methanol water extraction. The data revealed a statistically significant ($P < 0.05$) minimum inhibitory zone against *S.aureus, E.coli, MRSA and Candida albicans* when tested with the ethanol (soxhlet extraction), methanol (soxhlet extraction) and ethanol (water extraction) leaf extracts as compared to the negative controls. Furthermore, the results also indicated a statistically significant ($P < 0.05$) minimum inhibitory zone against fungi like *Aspergillus Flavus, Fusarium, Penicillium* evaluated with the ethanol, methanol (soxhlet extraction) and ethanol and methanol (water bath extraction) leaf extracts in comparison to the negative controls. In summary, our investigations demonstrated the potential of the *J. regia*'s leaf extracts as antimicrobial agents against disease causing bacteria and fungi.

Keywords: *Juglans regia*, Walnut, Antibacterial, Anti-fungal, leaf extracts, Organic extracts, antimicrobial activity, Natural Antibiotics.

1. Introduction:

Juglans regia Linn. (family, Juglandaceae), commonly known as English or Persian walnut deciduous tree, is popular for its exceptional nutritional value and potential medicinal properties against several diseases (Martínez et al., 2010). The genus *Juglans* was classified into different species and among them, *J. nigra* and *J. regia* were widely distributed and commonly found around the world. The walnut trees are mostly found in mountainous regions and most of the tree parts such as green walnuts, shells, kernels and seeds, bark and leaves, stems, pericarps, fruits, flowers and ligneous membranes are widely used in the pharmaceutical sector due to their medical properties (Akbari et al., 2012; Cosmulescu & Trandafir, 2011; Tsao, 2010; Zhu et al., 2004). Traditionally, the leaves of *J. regia* were utilized as a natural remedy to treat bacterial and fungal infections. Besides, the leaves' extracts from *J. regia* were a good source for natural medicinal compounds for treating numerous diseases like venous insufficiency, hemorrhoids, hypoglycemia, diarrhea, anti-helminthic, anti-cancerous and fungal or microbial infections (Acquaviva et al., 2019; Hosseinzadeh et al., 2011; Jahanban-Esfahlan et al., 2019).

Recently it has been reported that there is a continuous increase in the incidence of Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-resistant *Staphylococcus epidermidis* (MRSE) that are associated with various unpleasant conditions for the human health and has become a great challenge for their medical management with the existing therapeutic approaches (REF, REF). It is well documented that bioactive components isolated from the natural products have been evaluated for their therapeutic effects against a wide range of infectious microorganisms. Such phytochemicals have varied structural diversity that enables them to have effective biological functionality which could be essential for consideration of potential leads in drug discovery and designing (Rahman et al., 2013; Thomas et al., 2020).

Previous reports indicated that the leaves of walnut as a natural product received tremendous attention due to their biological activity as well as possessing omega-3 fatty acids (PUFA) as nutrients among other beneficial ingredients. Further, the leaf extracts were used for wound healing due to the high presence of tannins. They are highly effective in covering and protecting the skin from itching and inflammation when applied topically, specifically for gentle and shallow skin inflammation and over the top perspiring of the hands and feet. Likewise, the leaf extract from *J. regia* was applied on the skin and scalp suffering from sunburns and

stripping and tingling due to dandruff, respectively in France. It was also used as natural product to treat mellow skin condition as well as anti-parasitic and insecticidal agent (Kılıç et al., 2019; Wholehealth Chicago, 2009).

J. regia is well known for its antibacterial activity against a number of Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA)* and *E.coli.* The antibacterial effect of the walnut could stem from the fact that it has many bioactive chemicals ranging from juglone, regiolone, *β*-sitosterol, folic acid, gallic acid, quercetin-3-*α*-L-arabinoside, ascorbic acid etc.,(Zakavi et al., 2013). Hence, the leaves from walnut tree were regarded for their potential pharmacological effects viz., anti-fungal, antibacterial, anti-malarial and anti-inflammatory effects. Besides, the efficacy of the leaves extracts as pharmacological agent were further enhanced by using Poly lactic-co-glycolic acid (PLGA) nanoparticle formulation against bacteria and fungi (Kocacaliskan et al., 2018).

In our study, the collection and usage of the leaves from walnut tree for evaluating their antibacterial and anti-fungal effects in the laboratory setup were hassle-free due to the easy and ready availability from the Kurdistan region. Recently, there is a steady rise in the number of research groups including ours that are reporting the bioactive from plant, freshwater algae resources in Kurdistan region and their potential antimicrobial activities (Noel et al., 2021). The aim of the current study was to evaluate the antimicrobial properties of the leaves from *J. regia.* We tested our hypothesis whether the extracts in various solvents such as ethanol, methanol and water differ in their potential antimicrobial effects. Moreover, we further compared the antimicrobial activity based on the method of extraction using walnut leaves (Soxhlet's extraction *vs* water extraction) in water, ethanol and methanol. The antimicrobial effects of the extracts were assessed against different types of bacteria and fungi using the conventional minimum inhibitory zone assay.

2. Materials and methods

2.1. Chemicals

Nutrient agar (product code: 70148), Mueller Hinton agar (product code:70191), methanol, ethanol, and petri dishes were purchased from Sigma Aldrich Inc., USA.

2.2. Bacterial and fungal strains

Bacterial species including *Staphylococcus aureus, Methicillin-resistant S. aureus (MRSA), Pseudomonas aeruginosa, Pseudomonas aeruginosa (MDR), Klebsiella pneumonia, Klebsiella pneumonia (MDR), Escherichia coli* and fungi like *Candida albicans* and *Aspergillus niger* obtained from -------- were used to evaluate the antibacterial and anti-fungal effects of the leaf extracts, respectively.

2.3. Collection and preparation of J. regia leaf extract

The leaves from *J. regia* were collected freshly (month and year), and dried at room temperature. The plant material was authenticated by a taxonomist at our department and a sample was stored in our department herbarium for future reference (voucher no:). The dried leaves were powdered using sterilized mortar and pestle. 50 g of powder was used to prepare an organic extract in 150 ml of 100% ethanol, methanol and aqueous extract in double distilled water for one hour using Soxhlet's extractor. Further, the rotary evaporator was used in order to concentrate the aqueous and organic extracts and the final condensate was collected as pure extract samples. The samples were tested for their antimicrobial effects against selected bacterial and fungal species.

2.4. Culture and evaluation of antibacterial and anti-fungal activities

In order to obtain a standard inoculum with a density of 10^6 colony-forming units (CFU)/mL, we inoculated a loop of bacteria into 5 mL of nutrient broth and incubated at 37 °C for 24 h. After incubation, 200 µL of bacterial culture was added into 20 mL of nutrient broth and incubated further for 5 h for a standard culture of 10^6 CFUs/mL according to 0.5 McFarland turbidity standard test. The plates were prepared immediately with the standardized inoculums. The wells were made with 9 mm borer in bacteria seeded agar plates. An amount of 100 µL of walnut leaf extract was added in each well. The plates were incubated at 37 °C for 24 h. After incubation period the zone of inhibition was measured as reported previously (E. Abalaka et al., 2012).

Similarly, the fungal inoculums were prepared by adding the respective fungi samples into 2 mL of normal saline and vortexed for 1 min, 50 µL of the mixture was dispensed into the agar plates and incubated at 37 °C for 24-48 h. After, the well grown fugal agar plates were used for assessing the anti-fungal activity of the walnut leaf extracts. We defined the lowest concentration having no visible growth as minimum fungicidal growth (MFC) when there was 99.5% killing of the original inoculums (Suurbaar et al., 2017).

2.5. Statistical analysis

All the experiments were performed in triplicate for the statistical analyses. The results were represented as means ± standard error of mean. The group mean values of each experiment were compared by single-sided Student's *t*-test. The means were also compared by one-way analysis of variance and multiple pair-wise comparisons were done using the Tukey's test. $P < 0.05$ was considered to be the level of significance. Statistical analyses were performed using Graph Pad Prism 6 Software package for Windows.

3. Results (we should discuss results based on each figure)

In the present study, we utilized both Gram positive and Gram negative bacteria as well as Fungi in order to evaluate the potential antimicrobial activities of walnut leaf extracts in organic and aqueous solvents. Data revealed that the bioactivity of walnut leaf extracts depended on several factors like type of extraction methods (Soxhlet's vs water extraction), solvents (organic vs aqueous) and different kinds of bacteria and fungi. Interestingly, the leaf extracts from *J. regia* in ethanol and methanol by Soxhlet's extraction methods showed a statistically significant ($P < 0.05$) zone of inhibition with increasing concentrations against *S. aureus* (ethanol:10.5 ± 3.5 mm, methanol:14 ± 7.1 mm Fig. 1A, 2A), *E.coli* (ethanol:19 ± 4.5 mm, methanol: 21 ± 5.1 mm Fig. 1B, 2B), *MRSA* (ethanol: 20 ± 5.1 mm, methanol: 17 ± 4.1 mm Fig. 1C, 2C) and *C. albicans* (ethanol:20 ± 3.9 mm, methanol:19 ± 6.9 mm Fig. 1D) as compared to their respective controls.

In addition, the extracts by water extraction method in ethanol and methanol demonstrated a statistically significant (P <0.05) zone of inhibition with increase in concentrations against *S. aureus* (ethanol:14 ± 4.0 mm, methanol:19 ± 4.1 mm Fig. 1A), whereas with *E.coli*, it was only in water in ethanol but not in water in methanol (ethanol:15 ± 4.5 mm, Fig. 1B), *MRSA* (ethanol:15 ± 3.3 mm, methanol:17 ± 3.9 mm Fig. 1C) and *C. albicans* (ethanol:10 ± 4.9 mm, methanol:14 ± 3.9 mm Fig. 1D) when compared with their respective controls. However, the extracts obtained in water by Soxhlet's and water extraction methods did not reveal any statistically significant zone of inhibitions against *S.aureus, E.coli, MRSA* as well as *C. albicans* in comparison to their respective controls (Fig. 1A, 1B, 1C an 1D). Besides, the leaf extracts in methanol by water extraction methods showed no significant zone of inhibitions against *Pseudomonas aeruginosa, Pseudomonas aeruginosa* (*MDR*), *Klebsiella pneumonia, Klebsiella pneumonia* (*MDR*) when compared with the controls.

For anti-fungal effects, we tested the leaf extracts in water, ethanol and methanol by Soxhlet's and water extraction methods against *Aspergillus flavus, Fusarium* and *Penicillium.* Our findings divulged a significant anti-fungal effect against *Aspergillus flavus* from ethanol and methanol leaf extracts (Fig. 3C and 3D) as compared to the control groups (Fig. 3A). Nevertheless, the leaf extracts in water did not show any significant effect against this fungus (Fig. 3B) when compared to the control. We further evaluated the effects of the leaf extracts in water, ethanol and methanol against *Fusarium.* Data revealed a significant anti-fungal effect against *Fusarium* when the leaf extracts in water, ethanol and methanol were treated (Fig. 4B, C, D) as compared with the negative control (Fig. 4A). Besides, walnut leaf extracts in water, ethanol and methanol against *Penicillium* was assessed. The data showed a marked anti-fungal effect due to the leaf extracts in ethanol and methanol (Fig. 5C, D) but not in water (Fig. 5B) as compared to the no extract control.

4. Discussion

The current study focused on the effect of walnut leaf extract as a potential natural product with significant antibacterial and anti-fungal properties of . Moreover, the study gave emphasis to the kind of extraction methods in organic and aqueous solvents which would in turn determine the degree of bioactivity against a number of disease-causing bacteria and fungi. Our data demonstrated that the extraction of walnut leaf extract in ethanol and methanol by Soxhlet and water extraction methods had profound antibacterial and anti-fungal effects whereas the extract in water by Soxhlet and water extraction had no significant anti-microbial effects.. This phenomenon could be attributed the presence of higher content of phytochemicals such as tannins, polyphenols and flavonoids in the ethanol and methanol leaf extracts than in the water leaf extract. Our study was in agreement with previous report that the antioxidant activity exhibited by *J. regia* green husk extract was solvent specific (Zhang, 2015)and the type of extraction has influenced the content of phytochemicals and its anti-oxidant activity. The acetone, ethanol, and methanol extracts resulted in greater tannins, polyphenols and flavonoids with stronger antioxidant activities followed by ethyl-acetate and water extracts. Furthermore, the study concluded that the importance of obtaining fractions with potential anti-oxidant activity primarily depended on the selection of an appropriate solvent for the extraction from walnut green husk (Zhang, 2015).

Besides, a study explored *J. regia* dried leaves extracts along with *Camellia sinensis* (green tea) for antibiotic property against MDR strains (Farooqui et al., 2015). The study showed the synergistic effects of dried leaves extract from *J. regia* against MRSA when the extract was combined with oxacillin indicating the reversion of oxacillin resistance of MRSA strains *in vitro* (Farooqui et al., 2015). Our data also showed the antibiotic property of the leaf extracts from walnut against *S. aureus* as well as *MRSA* albeit without synergistic effects. Similarly several Gram positive (*Bacillus cereus, B. subtilis, Staphylococcus aureus*) and Gram-negative bacteria (*P. aeruginosa, E. coli, K. pneumoniae*) as well as fungi (*C. albicans, Cryptococcus neoformans*) were screened for antibacterial and anti-fungal properties of walnut leaves extract (Pereira et al., 2007). The data indicated selective inhibition of walnut leaves extract against *B. cereus* whereas Gram negative bacteria and fungi tested were resistant to the extracts even at highest concentration of 100 mg/mL. Furthermore, the antioxidant and antimicrobial properties of walnut (*J. regia* L.) green husks aqueous extracts from five different cultivars suggested dose-dependent antioxidant and bactericidal property against Gram positive bacteria, notably *S. aureus* was the highly prone bacteria with MIC of 0.1 mg/mL for all the extracts (Oliveira et al., 2008).

The leaves of walnut were found to contain higher amounts of phenolic compounds with excellent pharmacological and therapeutic properties. Moreover, they contain significant quantity of flavonoids that would confer them antibacterial, anti-fungal, antioxidant and among others (Einali et al., 2018; Jahanban-Esfahlan et al., 2019; Zhao et al., 2014). An extensive study by Raja et al., (Raja et al., 2017) demonstrated the anti-fungal effects of methanol extracts from roots of *J. regia.* They investigated nine different strains of *Candida* using MIC_{90} and spot assays. The root extract with effective anti-fungal activity in liquid media had MICs in the range of 300 to 700 μg/mL and *Candida* was highly susceptible to the *J. regia* root extract when studied using spot assay. Moreover, the gas chromatography-mass spectrometry analysis (GC-MS) analysis revealed greater presence of polyphenols, alkaloids, steroids, saponins, and tannins which would possibly be responsible for the bio-activity of *J. regia* root extract.

5. Conclusions

In the current study findings, we inferred that walnut leaf extracts in organic and aqueous solvents when extracted by Soxhlet and water extraction had differential antibacterial and anti-fungal properties. The leaf extract from walnut was found to be potently effective against *S.aureus, E.coli, MRSA and Candida.* Moreover, the growth of *Aspergillus flavus, Fusarium, Penicillium* were also influenced by type of solvents and extraction methods used such as ethanol (soxhlet extraction), methanol (soxhlet extraction), ethanol (water extraction) as well as methanol (water extraction). Nevertheless, the study warrants further investigations regarding the chemical analysis and potential mode of action of phytochemicals which are present abundantly in the leaf extract for their anti-microbial features.

Acknowledgments

The authors would like to thank the Komar Research Center [KRC] of Komar University of Science and Technology, Sulaymaniah, Kurdistan Region, Iraq for providing the facilities to conduct the research study.

Conflict of interest: The authors declare no conflict of interest.

References

Acquaviva, R., D'Angeli, F., Malfa, G. A., Ronsisvalle, S., Garozzo, A., Stivala, A., Ragusa, S., Nicolosi, D., Salmeri, M., & Genovese, C. (2019). Antibacterial and anti-biofilm activities of walnut pellicle extract (Juglans regia L.) against coagulase-negative staphylococci. Natural Product Research, 9, 1–6. https://doi.org/10.1080/14786419.2019.1650352

Akbari, V., Jamei, R., Heidari, R., & Esfahlan, A. J. (2012). Antiradical activity of different parts of Walnut (Juglans regia L.) fruit as a function of genotype. Food Chemistry, 135(4). https://doi.org/10.1016/j.foodchem.2012.07.030

Cosmulescu, S., & Trandafir, I. (2011). Seasonal variation of total phenols in leaves of walnut (Juglans Regia L.). Journal of Medicinal Plant Research, 5(19).

E. Abalaka, M., Y. Daniyan, S., B. Oyeleke, S., & O. Adeyemo, S. (2012). The Antibacterial Evaluation of Moringa Oleifera Leaf Extracts on Selected Bacterial Pathogens. Journal of Microbiology Research, 2(2), 1–4. https://doi.org/10.5923/j.microbiology.20120202.01

Einali, A., Azizian-Shermeh, O., & Ghasemi, A. (2018). Phytochemical screening and antimicrobial activities of Periploca aphylla Decne, Persian walnut (Juglans regia L.) and oleander (Nerium indicum Mill.) Leaf extracts. Journal of Food Measurement and Characterization, 12(2), 1350–1359. https://doi.org/10.1007/s11694-018-9749-9

Farooqui, A., Khan, A., Borghetto, I., Kazmi, S. U., Rubino, S., & Paglietti, B. (2015). Synergistic antimicrobial activity of Camellia sinensis and juglans regia against multidrug-resistant bacteria. PLoS ONE, 10(2), e0118431-undefined. https://doi.org/10.1371/journal.pone.0118431

Hosseinzadeh, H., Zarei, H., & Taghiabadi, E. (2011). Antinociceptive, anti-inflammatory and acute toxicity effects of juglans regia l. leaves in mice. Iranian Red Crescent Medical Journal, 13(1), 27–33.

Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (juglans regia l.) fruit and tree. In Molecules (Vol. 24, Issue 11, pp. 2133-undefined). https://doi.org/10.3390/molecules24112133

Kılıç, S., Okullu, S. Ö., Kurt, Ö., Sevinç, H., Dündar, C., Altınordu, F., & Türkoğlu, M. (2019). Efficacy of two plant extracts against acne vulgaris: Initial results of microbiological tests and cell culture studies. Journal of Cosmetic Dermatology, 18(4), 1061–1065. https://doi.org/10.1111/jocd.12814

Kocacaliskan, I., Albayrakb, A., & Ilhan, S. (2018). Varietal differences in antimicrobial activities of walnut (Juglans regia L.) leaf extracts. Gaziosmanpasa Journal of Scientific Research, 7(3), 173–180.

Martínez, M. L., Labuckas, D. O., Lamarque, A. L., & Maestri, D. M. (2010). Walnut (Juglans regia L.): Genetic resources, chemistry, by-products. In Journal of the Science of Food and Agriculture (Vol. 90, Issue 12). https://doi.org/10.1002/jsfa.4059

Noel, V. T., Dlzar, D. G., A Salomy, M. D., Rawa, R. I., & Lanya, K. J. (2021). Antibacterial effects of the organic crude extracts of freshwater algae of Sulaymaniyah, Kurdistan Region, Iraq. Journal of Medicinal Plants Research, 15(4). https://doi.org/10.5897/JMPR2021.7117

Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food and Chemical Toxicology, 46(7), 2326–2331. https://doi.org/10.1016/j.fct.2008.03.017

Pereira, J. A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P. B., Ferreira, I. C. F. R., Ferreres, F., Bento, A., Seabra, R., & Estevinho, L. (2007). Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chemical Toxicology, 45(11), 2287–2295. https://doi.org/10.1016/j.fct.2007.06.004

Rahman, M. A., Imran, T. bin, & Islam, S. (2013). Antioxidative, antimicrobial and cytotoxic effects of the phenolics of Leea indica leaf extract. Saudi Journal of Biological Sciences, 20(3), 213–225. https://doi.org/10.1016/j.sjbs.2012.11.007

Raja, V., Ahmad, S. I., Irshad, M., Wani, W. A., Siddiqi, W. A., & Shreaz, S. (2017). Anticandidal activity of ethanolic root extract of Juglans regia (L.): Effect on growth, cell morphology, and key virulence factors. Journal de Mycologie Medicale, 27(4), 476–486. https://doi.org/10.1016/j.mycmed.2017.07.002

Suurbaar, J., Mosobil, R., & Donkor, A. M. (2017). Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of Ricinus communis against selected pathogens. BMC Research Notes, 10(1), 660-undefined. https://doi.org/10.1186/s13104-017-3001-2

Thomas, N. V., Jan, G. S., & Klan, R. F. (2020). Control of Opportunistic Bacteria Using Aqueous and Ethanol Extracts of Rosa damascena. International Journal of Microbiology Research, 12(2), 1776–1778.

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. In Nutrients (Vol. 2, Issue 12). https://doi.org/10.3390/nu2121231

Wholehealth Chicago. (2009, May 19). https://wholehealthchicago.com/2009/05/19/walnut-leaf/.

Zakavi, F., Golpasand Hagh, L., Daraeighadikolaei, A., Farajzadeh Sheikh, A., Daraeighadikolaei, A., & Leilavi Shooshtari, Z. (2013). Antibacterial effect of Juglans regia bark against oral pathologic bacteria. International Journal of Dentistry, 2013, 854765-undefined. https://doi.org/10.1155/2013/854765

Zhang, Q. (2015). Effects of Extraction Solvents on Phytochemicals and Antioxidant Activities of Walnut (Juglans Regia L.) Green Husk Extracts. European Journal of Food Science and Technology, 3(5), 15–21.

Zhao, M. H., Jiang, Z. T., Liu, T., & Li, R. (2014). Flavonoids in Juglans regia L. leaves and evaluation of in vitro antioxidant activity via intracellular and chemical methods. Scientific World Journal, 2014, 303878-undefined. https://doi.org/10.1155/2014/303878

Zhu, X., Zhang, H., & Lo, R. (2004). Phenolic compounds from the leaf extract of artichoke (Cynara scolymus L.) and their antimicrobial activities. Journal of Agricultural and Food Chemistry, 52(24). https://doi.org/10.1021/jf0490192.

Figure captions

Figure 1. The graphs depicting the antibacterial and anti-fungal effects of the organic and aqueous extracts from the leaves of *J. regia* extracted by Soxhlet's and water extraction methods. The graphs indicated similar significant antimicrobial effects against *S. aureus* (A), *E. coli* (B), MRSA (C) and *C. albicans* (D) due to the ethanol and methanol extracts prepared by Soxhlet's and water extraction methods except methanol extract in water extraction against *E. coli* (B). Significance levels: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs control. The data shown as mean ± SEM.

Figure 2. The images showing antibacterial activity of walnut leaf extracts in water, ethanol, and methanol against *S. aureus* (A), *E. coli* (B) and MRSA (C) compared to the controls.

Figure 3. The images showing antifungal activity of walnut leaf extracts in water (B), ethanol (C) and methanol (D) against *Aspergillus flavus* compared to control (A)

Figure 4. The images showing anti-fungal activity of walnut leaf extracts in water (B), ethanol (C) and methanol (D) against *Fusarium* compared to control (A).

Figure 5. The images showing anti-fungal activity of walnut leaf extracts in water (B), ethanol (C) and methanol (D) against *Penicillium* compared to control (A).

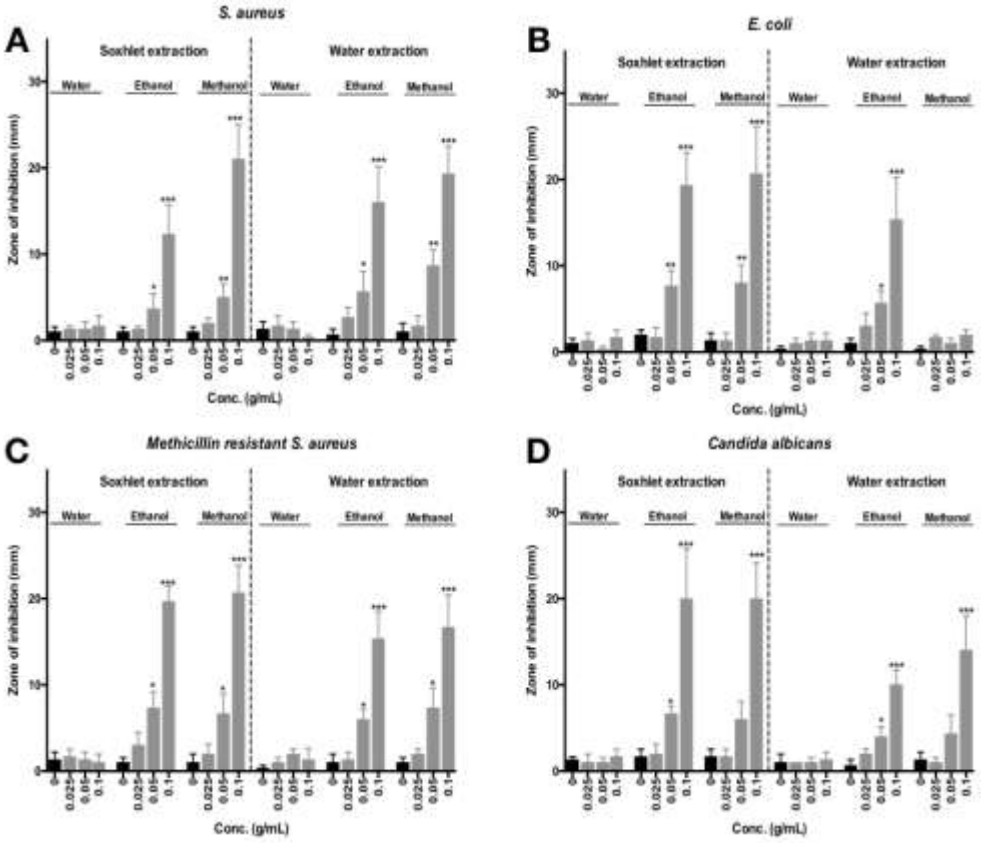


Figure. 1

Fig. 1. The graphs depicting the antibacterial and anti-fugal effects of the organic and aqueous extracts from the leaves of J. regia extracted by Soxhlet and water extraction methods. The graphs indicated similar significant antimicrobial effects against S. aureus (1A), E. coli (1B), MRSA (1C) and Candida albicans (1D) due to the ethanol and methanol extracts prepared by Soxhlet and water extraction methods expect methanol extract in water extraction against E. Coli (1B). Significance levels: * P<0.05, **P<0.01, *** P<0.001 vs control. The data shown as mean ± SEM.

Figure 2.

Fig. 2. The images showing antibacterial activity of walnut leaf extracts in water, ethanol, and methanol against S. aureus (2A), E. Coli (2B) and MRSA (2C) compared to the controls.

Figure 3.

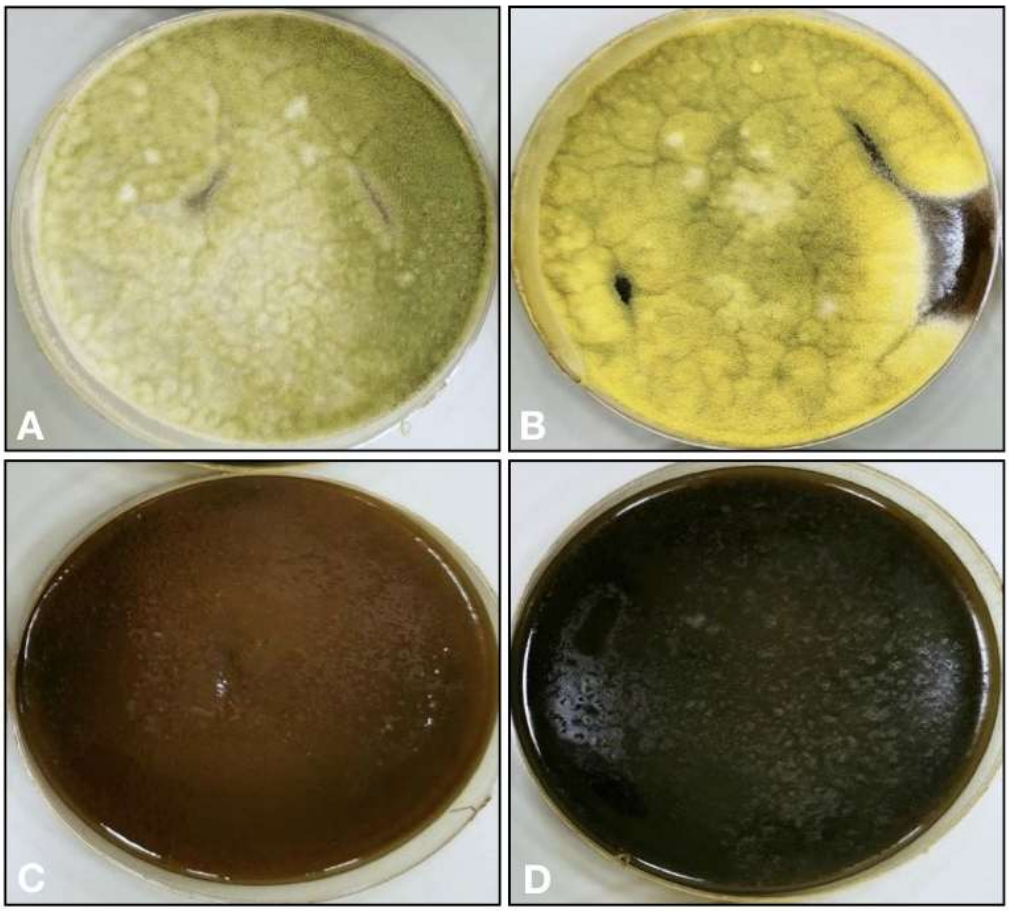


Fig. 3. The images showing anti-fungal activity of walnut leaf extracts in water (3B), ethanol (3C) and methanol (3D) against *Aspergillus flavus* compared to control (3A).

Figure 4.

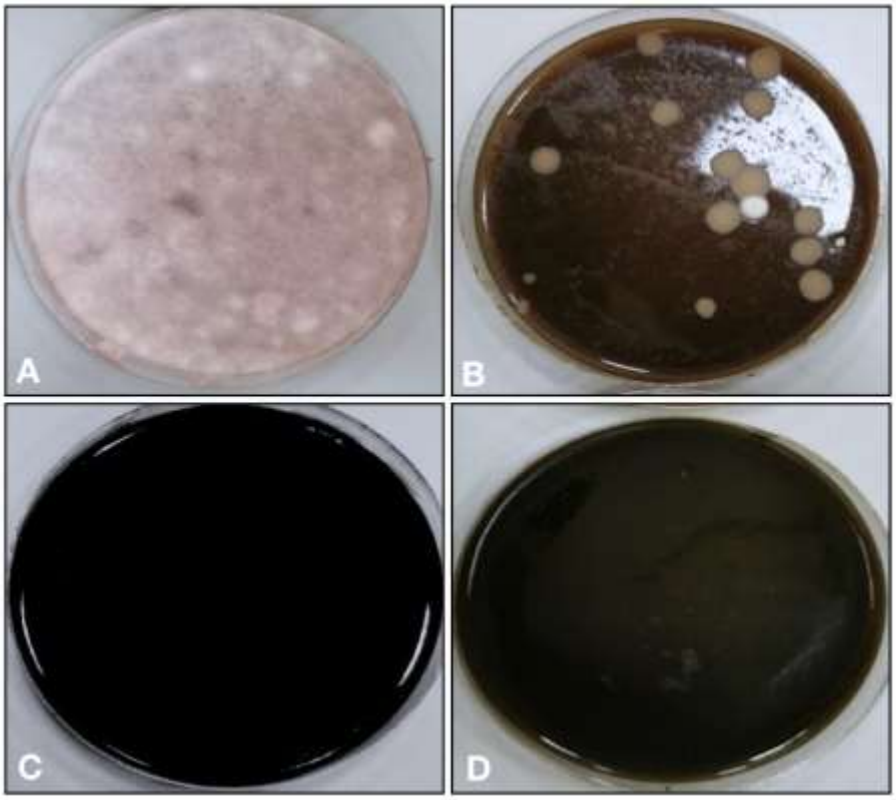


Fig. 4. The images showing anti-fungal activity of walnut leaf extracts in water (4B), ethanol (4C) and methanol (4D) against *Fusarium* compared to control (4A).

Figure 5.

Fig. 5. The images showing anti-fungal activity of walnut leaf extracts in water (5B), ethanol (5C) and methanol (5D) against *Penicillium* compared to control (5A).