Original article

Assessing the Effect of Walnut (*Juglans Regia*) and Olive (*Olea Europaea*) Oil Against the Bacterial Strains Found in Gut Microbiome

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Abstract. *Background and aim*: The problem of drug-resistance developed by bacteria against antibiotics turned the attention of researchers to find out and develop such products which haven't such issues. For this purpose, this study was carried out by using walnut and olive oil against several gram negative and gram-positive bacteria. *Methods:* Different concentrations of oils were employed using disc diffusion method. They exhibited better antibacterial properties. *Results:* All the tested bacteria, viz. *Citrobacter freundii*, *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium,* and *Shigella sonnei* were considered susceptible to certain concentrations of both walnut and olive oil. High bacterial activity (18.9±0.7 mm) of walnut oil was observed against *Salmonella typhi*, 17±1.2 mm against *Proteus vulgaris,* 16.90±1.5 mm against *Citrobacter freundii,* 15±0.8 mm against *Pseudomonas aeruginosa,* and 14±1.3 mm against *Klebsiella pneumoniae,* while average activity was 13±1.2 mm against *Salmonella typhimurium,* 12.90±1.5 mm against *Enterobacter aerogenes,* 12±1.5 mm against *Escherichia coli* and 12±1.00 mm against *Shigella sonnei.* Similarly, olive oil had its maximum antibacterial activity 26±1.5 mm, 23.1±1.00 mm, 19±1.5 mm, 19±1.5 mm, 19±0.8 mm, 18±1.1 mm, 18±1.5 mm, 15±1.00 mm, 15±0.7 mm, 14±1.1 mm against *Enterobacter aerogenes, Salmonella typhi, Citrobacter freundii, Escherichia coli, Shigella sonnei, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa,* respectively, whereas, optimum activity of olive oil was recorded against *Salmonella typhimurium* (13.15±1.0 mm). *Conclusions:* These findings showed that walnut and olive oils have a substantial effect on bacteria and can be used as effective antibacterial agents in the development of medicines.

Key words: antimicrobial activity, walnut oil, olive oil, bacteria, zone of inhibition

Introduction

The human microbiome is composed of bacteria, archaea, viruses, and eukaryotic microbes that reside in and on our bodies. These microbes have tremendous potential to impact our physiology, both in health and in disease. They contribute metabolic functions, protect from pathogens, educate the immune system, and, through these basic functions, affect directly or indirectly most of our physiologic functions (1) Gut microbiotas are integral to host digestion and nutrition, and they generate nutrients from substrates that are

otherwise indigestible by the host. The microbiota and the host immune system are numerous, complex, and bidirectional. The immune system must learn to tolerate the commensal microbiota and respond appropriately to pathogens, and in turn the microbiota is integral for educating the immune system to function properly (2). Disruptions to the microbiome have been associated with severe pathologies of the host, including , gastro diseases (3) metabolic diseases, cancer and inflammatory bowel disease (4). The need for development of newer antimicrobial chemotherapeutic agents is necessary, due to the increase in the failure rates of microbial infections treatment imposed by drug-resistant antibiotics. Certain pathogenic bacteria causes the imbalance in gut microbiome, which leads to various gastro intestinal diseases (5). The use of antibiotics against pathogenic bacterial diseases, not only cause drug resistance, but also disturbs the normal microbial flora of gastrointestinal tract and make the body susceptible to several intestinal diseases. Herbal extracts have natural origin, so are more compatible with body of organism as compared to antibiotics and their side effects are very rare (6). It is scientifically proved that the products isolated from traditional plants show its activity against such pathogenic microorganisms which are normally resistant to antimicrobial agents. These plant products showed its activity against broad range of microorganisms. The chemical composition of these plant antimicrobial agents and the mechanisms of microbial growth inhibition is described by many researchers using these extracts separately or in combination with other antimicrobials. Drugs can be developed and synthesize from these medicinal plants which are rich resources of such ingredients. In pharmaceutical industry the medicinal plants are playing an important role in research developments. In such researches the main aim is to find out the ways to isolate and develop active medicine, or to develop semi-synthetic drugs from these active compounds (7). Certain chemical compounds of these plants have medicinal value due to its physiological impact on the human body. Some important bioactive compounds of plants are alkaloids, tannin, flavonoid, and phenolic compounds. They exert an effective action against infectious agents without the typical side effects imposed by synthetic antimicrobials. So, the screening of such medicinal plants is very essential to isolate and employ their active ingredients in medicines, which can result in the development of new and substantial medicines against the drug-resistant microorganisms (8).

The walnut plant (Fig 1A) is widely distributed all over the world (9). Species of the walnut are cultivated throughout Asia, India, China, Europe, North America, North Africa, and East Asia. Walnuts are considered a highly nutritious food and have been used traditionally to treat cough, stomachache and cancer in Asian and European countries (10). The olive tree (Fig 1B)

Figure 1. (A) walnut *juglans regia* plant with its fruit. (B): olive *Olea europaea* plant with its fruit.

(*Olea europaea*) has been cultivated from ancient times in the Mediterranean area to produce table olives, olive oil and olive leaf extracts. The polyphenols from Olea europaea L. matrices (olive oil by-products, leaves and olive pulp) are known for their good antioxidant properties and protective biological and biomedical effects. The chemical characterization and quantitative evaluation of these minor polar compounds can be useful to obtain active principles with important applications in pharmaceutical, cosmetic, and functional food products. The main constituent of olive leaf is a phenolic secoiridoid glycoside, oleuropein, which can be broken down into elenolic acid, a powerful anti-bacterial molecule (11) and hydroxytyrosol (OH-Tyr), a small molecule well known for its strong antioxidant activity (12).The pharmaceutical and beauty industry use certain parts of walnuts, like green shells, kernel, seed, bark, and leaves in their products (13).

In some countries the bark of *Juglans regia* is used as a toothbrush and as a dye to beautify the lips. It has been reported that walnut plant can be used in various disease conditions as antibacterial, antifungal, antiviral activity, antioxidant, antidiabetic, anthelmintic, anti-inflammatory, antidepressant, anti-tyrosinase, hepatoprotective, hypotriglyceridemic & anticancer agents and has blood purify, depurative, diuretic, and laxative activities (14). It is necessity to find and develop new antimicrobial agents due to the increase in resistance to antibiotics, wide-spread use of immune-suppressing drugs and a rise in bacterial infections (15). An effective increase has been reported in the data from the last few years regarding the benefits of olive and its other products (16). Data has been shown that different types of cancer including intestine, prostate, breast, as well as other diseases neurological disorders, cardiovascular diseases and thrombotic diseases can be prevented by phenolic compounds of olive oil (17). *Juglans regia* leaves have been used mostly in worldwide traditional medicines as antimicrobial, anthelmintic, astringent, keratolytic, antidiarrheal, hypoglycemic, depurative, tonic, carminative, arthritis, asthma, eczema, scrofula, skin disorders and for the treatment of sinusitis, cold and stomachache. In Iran inflammatory bowel disease is treated by the kernel of *J. regia*. Walnuts have several essential nutrients of diet like fats, proteins, vitamins, and minerals. They also contain flavonoids, sterols, pectic substances, phenolic acids and related polyphenols due to which they have the medicinal characteristic (18).

This study was aimed to determine the antibacterial activity of walnut (*juglans regia*) and Olive (*Olea europaea*) oils against different bacterial strains that affects gut microbiota.

Materials and Methods

Plant materials

Walnuts were purchased from the local market of Chakdara, Dir (Lower), Khyber Pakhtunkhwa, Pakistan. The shells were removed, endosperm was collected and processed through cold pressing method to extract the oil. While pomace olive oil was purchased from the same local market of Chakdara.

Oil dilution:

Serial dilution method was used to prepare various concentrations from the walnut and olive oil. Both the oils were diluted in dimethyl sulfoxide (DMSO) to obtain different concentration. About 35 different concentrations were prepared from the oils

Bacterial strains collection:

Different bacterial strains affect gut microbiota were collected from Department of Pharmacy, University of Malakand, Genentech diagnostic and Research Laboratories, and Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan. These bacterial strains include gram negative bacteria.

Test organisms:

The bacteria collected and used in this study are listed in Table 1.

Culturing of bacterial strains:

The bacterial strains were cultured in nutrient broth. The composition of the nutrient broth (100 ml) prepared

Table 1. List of bacterial strains used.

S. No	Bacterial Strain
1	*Citrobacter freundii*
2	*Enterobacter aerogenes*
3	*Escherichia coli*
4	*Salmonella typhi*
5	*Proteus vulgaris*
6	*Salmonella typhimurium*
7	*Shigella sonnei*
8	*Vibrio cholerae*

for culturing was Peptone 1.5 gm, NaCl 0.6 gm and Yeast extract 0.3 gm. These components were dissolved properly in 30ml distilled water in a sterile environment and then raised the volume to 100 ml. The dissolved media was sterilized at 121 °C for 20 minutes in autoclave. The media was then poured into the test tubes under the sterile environment of laminar airflow to avoid any contamination. Then the media was inoculated with the bacterial strains collected earlier and the test tubes were incubated overnight at 37 °C for growth.

Culturing of bacteria on petri plates:

Nutrient agar medium was prepared to culture the bacteria on petri plates. The medium was dissolved, autoclaved, and then poured in plates under the setup of laminar airflow. The media in the plates were then allowed to solidify. After some time, media solidified. Then the bacteria cultured in broth were taken and the petri plates were inoculated by the bacteria using sterile cotton swabs. The bacteria were thoroughly spread on each plate with sterilized swabs for uniform distribution. Then the inoculated plates were placed in the incubator at 37 °C for 24 hours. After 24 hours, the bacterial colonies were observed on every plate.

Use of walnut and olive oil to assess their antibacterial effect

Disc diffusion method was used to evaluate the antibacterial effect of walnut oil. About 6 mm diameter discs were used which had the capacity to absorb about 10 µl solution. These discs were placed with the help of forceps on the petri plates having bacterial growth. Then 10 µl of different concentrations of oil were applied to each disc with the help of pipette and allowed to dry. Similarly, standard antibiotic discs including *sulphonamides, clindamycin, norfloxacin, cefixime, mupirocin, tetracycline,* etc were applied to the plates as positive control. All this process was performed in laminar airflow machine to avoid contamination in the work. The petri plates were transferred to the incubator and stored at 37 °C for 24 hours.

The antibacterial activities of the oils were observed after 24 hours of the incubation time of plates in incubator. The zones of inhibition produced by different concentrations of oil were measured in triplicates to determine the effectiveness of the oils against various bacteria.

Statistical analysis

All the results recorded from the study were analyzed statistically. Mean and standard deviation of the values along with comparative graphs of both the oils were determined using GraphPad Prism 5 software.

Results

Antibacterial activity of walnut oil and olive oil against Citrobacter freundii:

The antibacterial activity of different concentrations of walnut oil and olive oil was determined against *Citrobacter freundii*. The results have been shown in Figure 2. The maximum activity of the walnut oil was observed at the concentration of 4.5 µg and the zone of inhibition was 16.9±0.8 mm, while the maximum activity of olive oil was at the concentration of 8 µg and the zone of inhibition was 19±0.6 mm. When the concentration was further increased from 4.5 µg in case of walnut oil and 8 µg in case of olive oil, there was no further increase in antibacterial activity (Figure 2). The minimum activity of walnut oil was observed at 6 µg having the zone of inhibition of 6±0.5 mm, while there was no activity at 4, 7, 13,

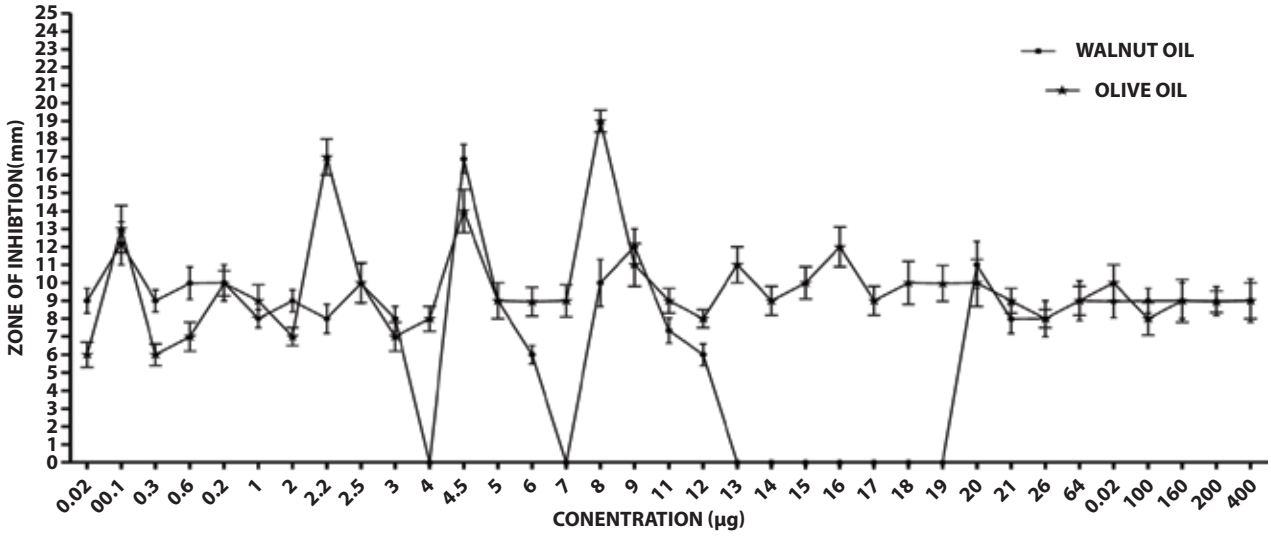

Figure 2. Antibacterial activity of walnut oil and olive oil against *citrobacter freundii.*

14, 15, 16, 17, 18, and 19 µg concentrations. The least activity of olive oil was observed at 0.02 µg having the zone of inhibition of 6±0.7 mm. It has been found that walnut oil and olive oil may be used against *C. freundii*, as both have better activities at low concentration against the said bacteria.

Antibacterial activity of walnut oil and olive oil against Enterobacter aerogenes

The antibacterial activity of walnut oil and olive oil was determined at different concentrations against *Enterobacter aerogenes* (Figure 3). Maximum activity of the walnut oil was found at the concentration of 2.2 µg having the zone of inhibition of 12.9±1.5 mm, while the olive oil has shown its maximum activity at concentration of 20 µg having the zone of inhibition of 26±1.5 mm. When the concentrations of oils further increased from 2.2 µg in case of walnut oil and 20 µg in case of olive oil, there was no further increase in antibacterial activity as shown in Figure 3. Walnut oil had shown minimum activity at 80 µg having the zone of inhibition 6±0.5 mm, while at 2, 3, 6, 7, 11, 12, 14, 15, 16, and 17 µg concentrations no activity had been recorded. Minimum activity of olive oil was observed at 2 µg having 6±0.8 mm zone of inhibition, while there was no activity at 0.1 µg concentration.

Antibacterial activity of walnut oil and olive oil against Escherichia coli:

Different concentrations of walnut oil and olive oil were used to determine the antibacterial activity against *Escherichia coli* and the results have been shown in Figure 4. Highest activity of the walnut oil was observed at the concentrations of 9 and 400 µg having the zones of inhibition of 12±1.5 and 12±1.4 mm, respectively. The maximum activity of olive oil was observed at concentration of 20 µg as the zone of inhibition was 19±1.5 mm. When the concentration was further increased from 9 µg (except 400 µg) in case of walnut oil and 20 µg in case of olive oil, there was no further increase in antibacterial activity as shown in Figure 4. The minimum activity of walnut oil was observed at 14 µg having the zone of inhibition of 6±0.9 mm, while there was zero activity at 2, 3, 4, 6, 7, 11, and 12 µg concentrations. The minimum activity of olive oil was recorded at 18 µg having the zone of inhibition of 7.95±0.5 mm. It has been found that olive oil has maximum activity against *E. coli* as compared to walnut oil. Therefore, olive oil may be recommended in case of *E. coli* infection.

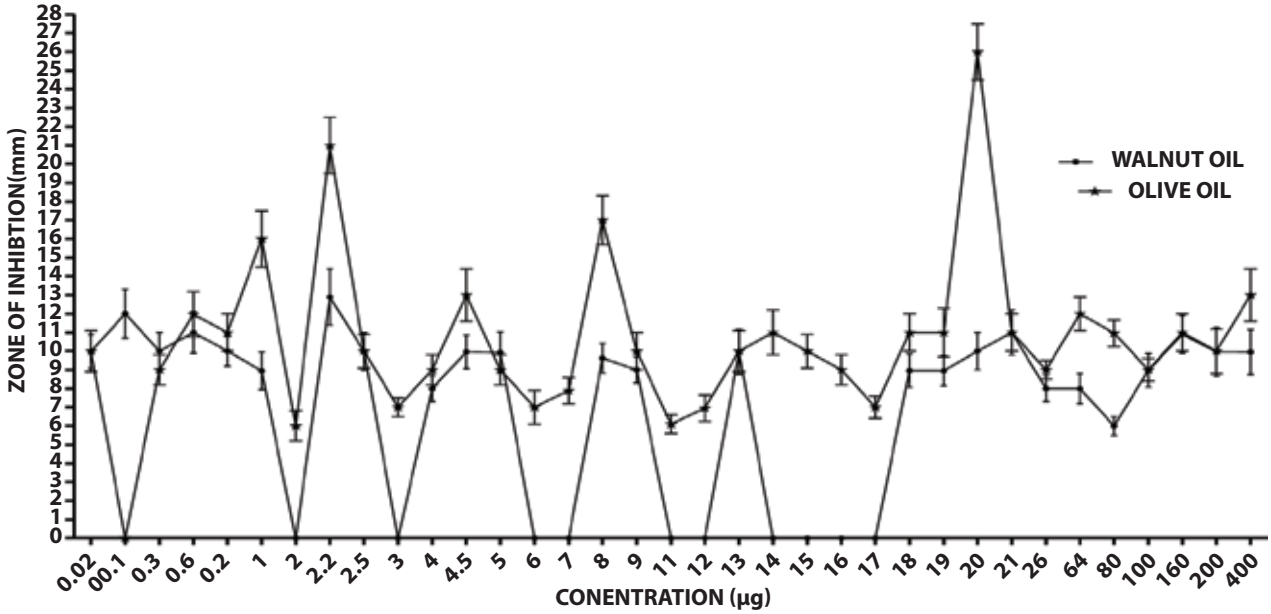

Figure 3. Antibacterial activity of walnut oil and olive oil against *Enterobacter aerogenes.*

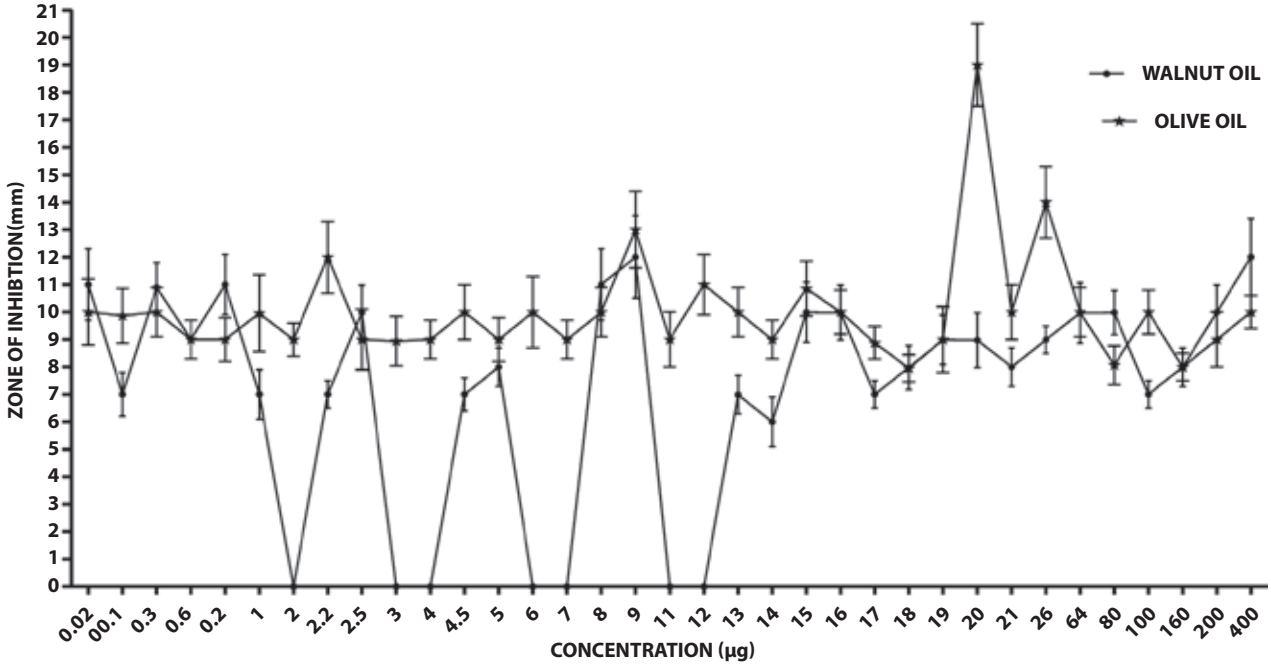

Figure 4. Antibacterial activity of walnut oil and olive oil against *Escherichia coli.*

Antibacterial activity of walnut oil and olive oil against Proteus vulgaris:

Various concentrations were used to determine antibacterial activity of walnut and olive oil against *Proteus vulgaris,* and the results obtained have been mentioned Figure 5. At 2.2 µg concentration of walnut oil, the zone of inhibition was 17±1.2 mm, while maximum antibacterial activity was recorded for olive oil at 9 µg and its zone of inhibition was 15±0.7 mm.

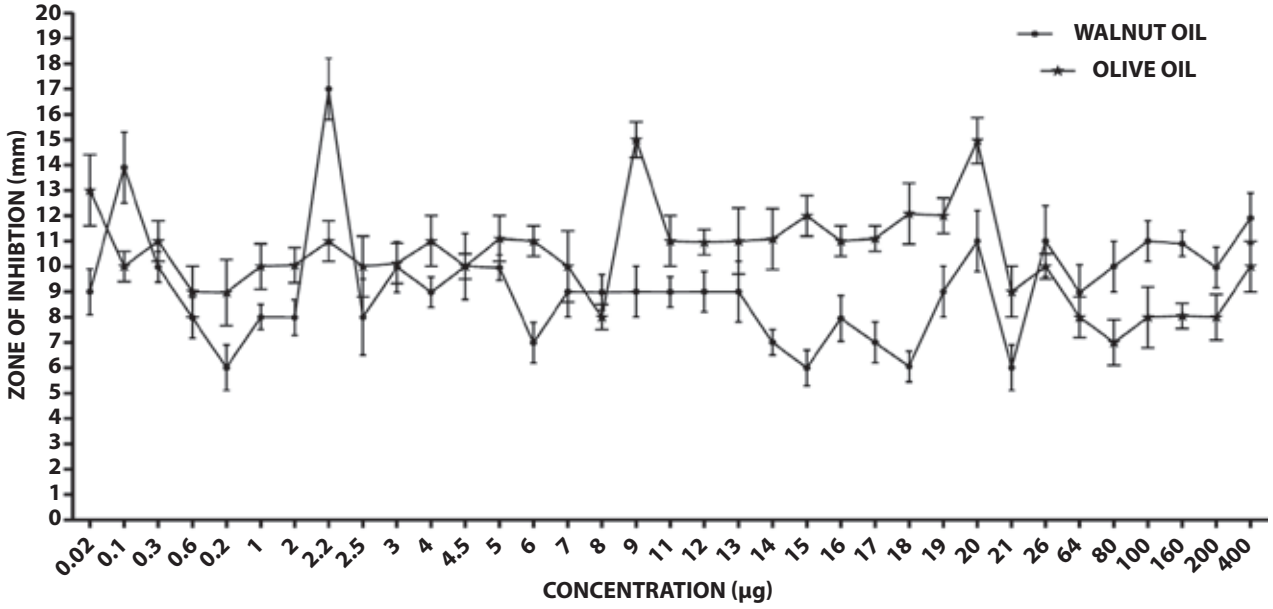

Figure 5. Antibacterial activity of walnut oil and olive oil against *Proteus vulgaris.*

At 15 µg concentration of walnut oil, lowest activity was observed having zone of inhibition of 6±0.7 mm and olive oil had given its lowest value at 80 µg as zone of inhibition of 7±0.9 mm. It has been observed that in case of *Proteus vulgaris*, walnut oil showed better activity at low concentration as compared to olive oil, therefore, walnut oil may be used against *Proteus vulgaris.*

Antibacterial activity of walnut oil and olive oil against Salmonella typhi:

Different concentrations of walnut and olive oil have been mentioned in Figure 6 along with zones of inhibition against *Salmonella typhi.* From the results, it has been concluded that walnut oil had its maximum activity of 21 µg giving the zone of inhibition of 13±1.2 mm, while olive oil had its maximum zone of inhibition of 13.15±1 mm at 0.3 µg. The least antibacterial activity of walnut and olive oil was recorded at 6 µg having zone at inhibition of 6±0.7 mm and at 4 µg giving the zone of inhibition of 7.95±1 mm, respectively. At 2, 4, 4.5, 9, 11, 12, and 19 µg of walnut oil, no antibacterial activity was observed. Olive oil showed high activity against *S.typhi* at lowest concentration, therefore may be used against *S. thyphi*.

Antibacterial activity of walnut oil and olive oil against Salmonella typhimurium:

Against *Salmonella typhimurium,* various concentrations of walnut and olive oil were used to determine their antibacterial activity (Figure 7). At concentration of 20 µg walnut oil had the zone of inhibition of 18.9±0.7 mm and at 0.1 µg, olive oil had the zone of inhibition of 23.1±1 mm. Walnut oil had given its minimum activity at 11 µg having the zone of inhibition of 6±1 mm, while olive oil had its least zone of inhibition (8±0.5 mm) at 26 µg. When the concentrations were further increased or decreased from these concentrations there were not any effective activities observed. Olive oil showed high activity against *S. typhimurium* at lowest concentration and may be used as a treatment against *S. typhimurium* infection.

Antibacterial activity of walnut oil and olive oil against Shigella sonnei:

Figure 8 shows the antibacterial activity of walnut and olive oil at various concentrations against *Shigella sonnei.* The walnut oil had its maximum activity at 0.3 µg having zone of inhibition of 12±1 mm, while maximum activity of olive oil had observed at 9 µg concentration, as

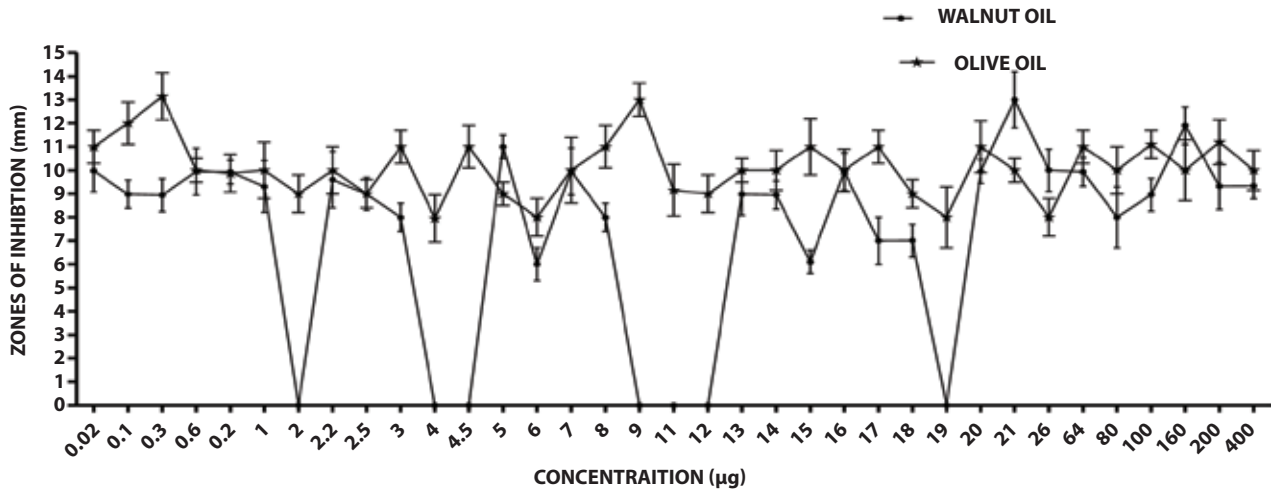

Figure 6. Antibacterial activity of walnut oil and olive oil against *Salmonella typhi.*

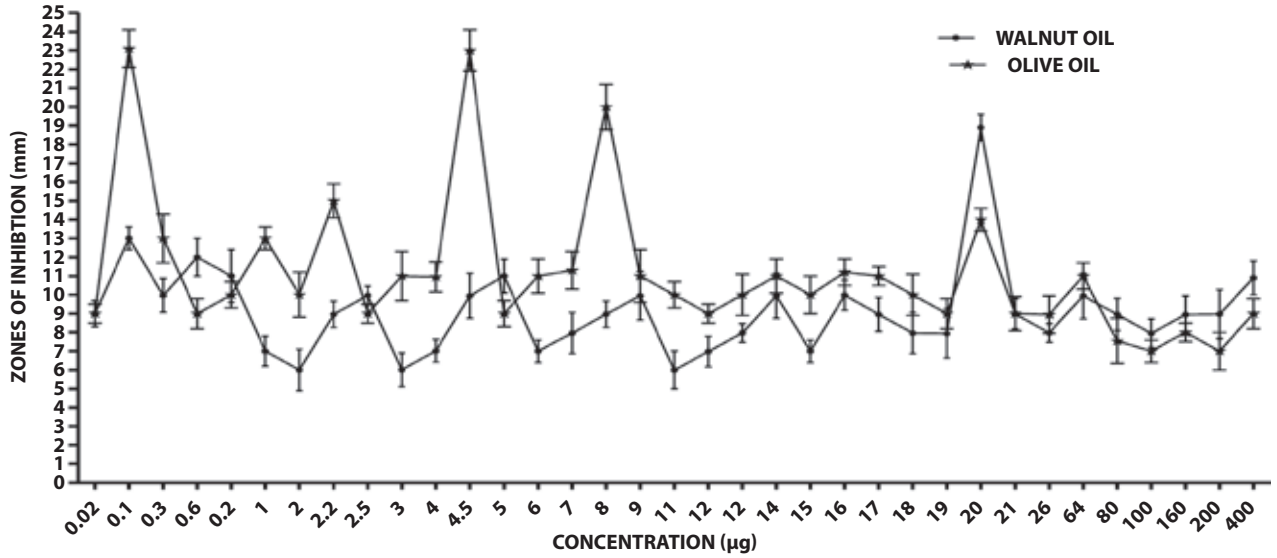

Figure 7. Antibacterial activity of walnut oil and olive oil against *Salmonella typhimurium.*

zone of inhibition of 19±0.8 mm. The lowest antibacterial activities shown by walnut and olive oil were at 17 µg and 100 µg concentrations, where the zones of inhibition were 6±0.8 and 6±0.6 mm, respectively. At concentrations of 3, 4, 6, 7, 14, 15, and 19 µg of walnut oil and 0.1 µg of olive oil, there was zero antibacterial activity.

Antibacterial activity of walnut oil and olive oil against Vibrio cholerae:

Various concentrations of walnut oil and olive oil were checked for the antibacterial activity against *Vibrio cholerae.* The results against the said bacterium have been mentioned in graph no 3.13. It was observed that walnut oil had maximum antibacterial activity at 80 µg and its zone of inhibition was 14±0.6 mm, while olive oil had maximum activity at 16 µg concentration as of inhibition of 11±1.2 mm. Walnut oil and olive oil had the lowest zones of inhibition of 6.96±0.7 mm and 7±0.7 mm at 0.6 µg and 4 µg concentrations, respectively. No antibacterial activity seen at 2, 4, 4.5, 6, 7, 11, 12, 14, and 15 µg of walnut oil and at 0.1, 1, 2.2, 4.5, 8, 9, and 20 µg concentrations of olive oil. It has been found that olive oil had comparatively better

activity than walnut oil against *Vibrio cholera*e but having the low diameter of zone as shown in Figure 9.

Discussion

In current study, walnut and olive oils were used against Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Proteus vulgaris, Salmonella typhimurium, Shigella sonnei, Vibrio cholera. Disc diffusion method was used to check the antibacterial activity of both olive and walnut oils. Both oils showed inhibitory zones at various concentrations against the test organisms.

Walnut oil against Citrobacter freundii

Different concentrations of walnut oil were used against *Citrobacter freundii*. The highest growth inhibition of bacteria was recorded (16±1.5 mm zone) at concentration of 4.5 µg. No evidence of such work has been present in the literature.

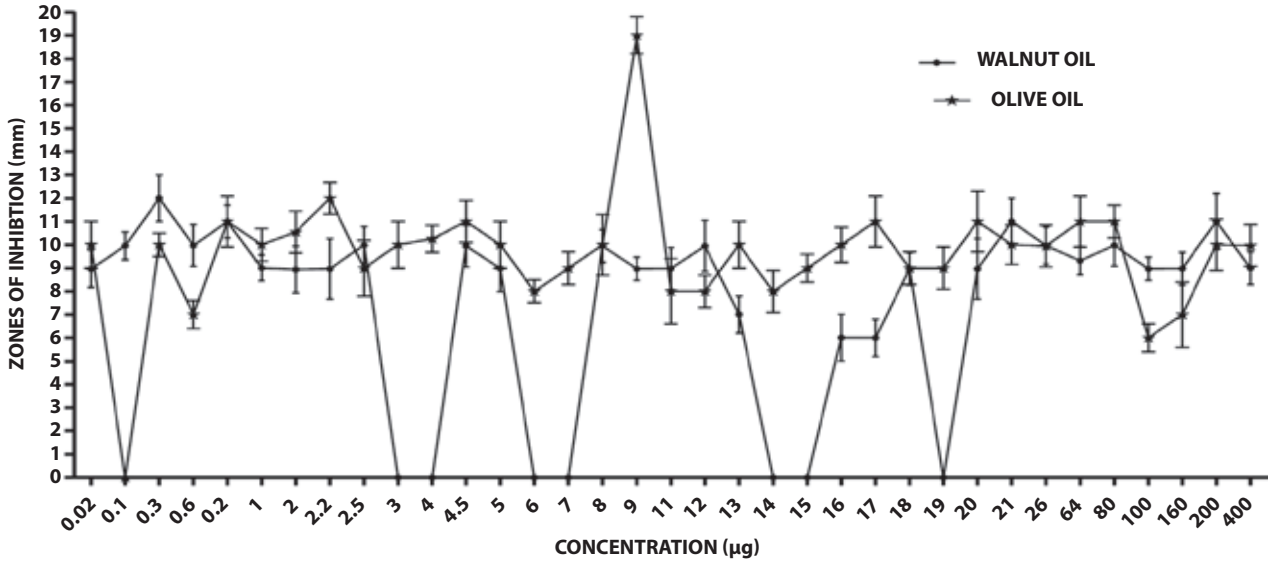

Figure 8. Antibacterial activity of walnut oil and olive oil against *Shigella sonnei.*

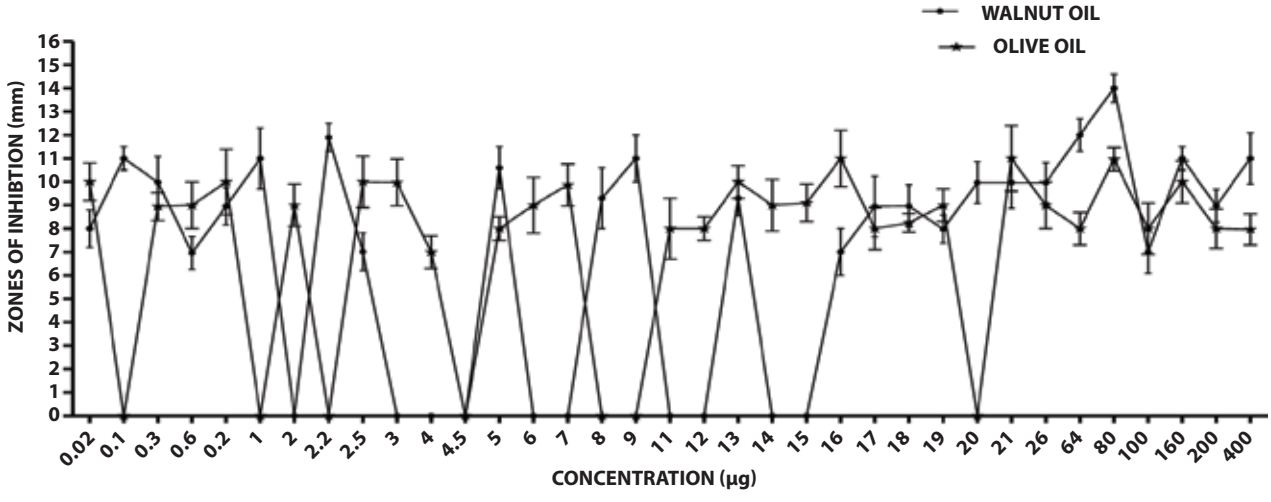

Figure 9. Antibacterial activity of Walnut oil and Olive oil against *Vibrio cholerae.*

Olive oil against Citrobacter freundii

In the current study, *Citrobacter freundii* was used to check the antibacterial activity of olive oil and maximum zone of inhibition was recorded (19±0.6 mm) at 8 µg concentration. Likewise, a study was performed by Hussain et al., (19) in Pakistan, using petroleum ether, chloroform, ethanol and methanol crude extracts of wild olive stem and root bark against several bacteria. There is deviation from present study because the antibacterial activity against *Citrobacter freundii* was different having different zones of inhibition. The zones of inhibition of stem bark extract (10 mg/ml) in petroleum ether, chloroform, ethanol, and methanol are 0.00±0.00, 14.50±0.50, 20.50±0.50 and 22.16±0.28 mm, respectively. Similarly, root bark petroleum ether, chloroform, ethanol, and methanol extracts (10 mg/ml) had 0.00±0.00, 12.16±0.28, 30.50±0.50 and 29.50±0.50 mm inhibition zones, respectively. This deviation may be due to the use of different extraction solvents and different parts of plant, which might have different effects against *Citrobacter freundii*.

Walnut oil against Enterobacter aerogenes

Walnut oil was used against *Enterobacter aerogenes* and a better activity against the bacterium was observed. The maximum zone of inhibition was 12.9±1.5 mm at 2.2 µg concentration. No study regarding the mentioned bacterium has been found in the literature.

Olive oil against Enterobacter aerogenes:

The growth inhibition of Enterobacter aerogenes by olive oil was far better in the current study because 26±1.5 mm ZOI was recorded at the concentration of 20 µg. In previous work performed by researchers, no such activity or record has been mentioned in the case of the said bacterial strain.

Walnut oil against Escherichia coli

In present study, walnut oil was used against *Escherichia coli* and its activity was better against the said bacterium. The highest antibacterial activity of the walnut oil was observed at 9 µg and 400 µg as ZOI were 12±1.5 and 12±1.4 mm, respectively. The results obtained by Ajaiyeoba et al., (5) are in agreement with the present study, who evaluated antibacterial and antifungal activities of the leaf, stem bark, kernel and root methanol extracts and also the hexane, chloroform, ethyl acetate and methanol fractions of leaf of the African walnut, using the agar cup diffusion and agar broth dilution techniques. The study of Taha et al., (18), mentioned that the antimicrobial activity against gram-negative bacteria was selective since not all the fruit extract of *J. regia* cultivars inhibited the growth of *Pseudomonas aeruginosa* and *E. coli*, while some cultivars had inhibited the growth of the said bacteria. The MIC mentioned by them for *E. coli* was 10 mg/ml. The same study was performed by Orhan et al., (20) who has observed significant activity of the said oil against American type culture collection (ATCC) *E. coli* strain at 4 µg/ml and isolated strain at 16 µg/ml concentration, which showed deviation from this study. The medicinal properties of walnut oil were also tested by Rathi et al., (21) which showed a little deviation from the current study. It has been found that it had antibacterial activity against *Staphylococcus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris*, where the zone of inhibition of *E. coli* was 14 mm, while the ZOI in the present study was 12±1.5 mm. Deviation was also reported from the present study in an evaluation carried out by Joseph et al., (22) testing walnut oil and turmeric oil for its antibacterial activity against *E. coli* and S. aureus. They concluded that walnut oil had no inhibitory activity against *E. coli*. Another study performed using walnut oil also showed deviation from our findings because, Saxenaa et al., (23) observed no antibacterial activity against *Escherichia coli*. The reported deviation may be due to the difference in geography, the methods of extraction used, or the bacteria were resistant to the walnut oil used by them in their study.

Olive oil against Escherichia coli

Escherichia coli was also used to test the antibacterial activity of olive oil and the end results obtained reveal the better activity of the said oil. Antimicrobial, antioxidant and anti-inflammatory activities in extra virgin olive oil was determined by Cicerale et al (24)

and the results obtained for antimicrobial activity were in agreement with the present study regarding *E. coli.* Ali Ahmad (25), had also evaluated the antibacterial activity of olive leaves extract (OLE) against several bacteria including *E. coli* and there was a good activity against the mentioned bacterium. The study performed by Pereira et al (26) showed deviation from present study, in which extracts of olive leaves were screened for the antimicrobial activity against *B. cereus, B. subtilis, S. aureus, E. coli, P. aeruginosa and K. pneumoniae* and few species of fungi. The deviation showed in the activity may be due to the reason that they used olive leaves extract while in present study olive fruit oil was used to check the antibacterial activity.

Olive oil against Proteus vulgaris:

Olive oil was also tested in this study for its antibacterial activity against *Proteus vulgaris* and the activity was better regarding the mentioned bacterium. A study was conducted using virgin olive oil samples from three cities of Turkey by Dağdelen (17) to evaluate the antioxidant and antimicrobial activities against various bacteria. It was reported that the said oil samples from Edinick, Gomec and Izmir cities had 7.00±0.00, 7.33±0.58 and 9.33±0.58 mm activity against *Proteus vulgaris*, respectively, while in the present study better result was observed (15 mm at 9 µg). This deviation in the activity may be due to the difference of geography or susceptibility of the bacterial strains used.

Walnut oil against Salmonella typhi:

In the present study much, better results were observed when walnut oil was used to check its antibacterial potential against *Salmonella typhi.* At 21 µg of walnut oil, 13±1.2 mm ZOI was observed for this bacterium. No data regarding walnut oil against the mentioned bacterium has been reported in literature.

Olive oil against Salmonella typhi:

The current study was conducted using olive oil against *Salmonella typhi* to check the antibacterial activity of the said oil and showed better activity having 13.15±1.00 mm zone of inhibition at 0.3 µg. (25), who had used crude olive leaves alcoholic extracts against several bacteria checking the decrease in bacterial counts in samples of peeled undeveined (PUD) shrimp, is in agreement with the present study. The used extracts had inhibited the growth of *Salmonella typhi*.

Walnut oil against Salmonella typhimurium:

Walnut oil was used in current study against *Salmonella typhimurium* and the results obtained were better as the ZOI was 18.9±0.7 mm at 20 µg concentration. The study of (18) has mentioned that walnut oil had a significant antibacterial activity against *Salmonella typhimurium*, which is in agreement with the current study. In the study of (23) using several bacteria and reported no antibacterial activity against *Salmonella typhimurium*, which showed deviation from the present study. This deviation may be due to the use of resistant bacteria in their study.

Olive oil against Salmonella typhimurium:

A better antibacterial activity of olive oil was observed against *Salmonella typhimurium* in the present study. The study performed by (17) using virgin olive oils from three different areas in Turkey against different bacteria showed deviation from the present study. His study revealed the following antibacterial activity against *Salmonella typhimurium*; Edincik oil 11.67±0.58, Gomec oil 7.33±0.58 and Izmir oil 8.33±0.58 mm. The deviation in the study may be due to the use of virgin olive oil extracted by phenol in his study.

Walnut oil against Shigella sonnei:

This study was performed to analyze the antibacterial properties of walnut oil against *Shigella sonnei.* The growth inhibition results were better against the said bacterium in this study as the maximum zone of inhibition was 12±1 mm at 0.3 µg.

Olive oil against Shigella sonnei:

In the present study the antibacterial activity of olive oil was evaluated against *Shigella sonnei* and

inhibited its growth. A study in which several edible oils, including olive oil, were tested by (27) against various bacteria and they evaluated that inhibition of most of the bacteria occurred after one hour contact with olive oil and only the two Gram-negative bacteria, *S. sonnei* and *E. coli*, partly survived. (17) also has deviation, who mentioned least antibacterial activity of virgin olive oil against *Shigella sonnei*. The zones of inhibition were 10.67±0.58 mm (Edinick phenolic extract), 8.67±0.58 mm (Gomec phenolic extract) and 8.67±0.58 mm (Izmir phenolic extract), while in our study that was 19±0.8 mm. Difference in oil samples used or susceptibility of the bacteria to the samples may be the reason of deviation in both the studies.

Walnut oil against Vibrio cholerae:

Vibrio cholerae was used as test bacterium in this study to observe the bacterial inhibition of walnut oil and at 80 µg walnut oil, there was 14±0.6 mm inhibition zone. No reported data is available regarding this parameter.

Olive oil against Vibrio cholerae:

Different concentrations of olive oil were used against *Vibrio cholerae* showed a better antibacterial activity. Similarly, Bisignano et al., (28) used ATCC and clinically isolated strains of several bacteria to evaluate the antibacterial properties of the components (oleuropein and hydroxytyrosol) of olive oil. They concluded that these two components, oleuropein and hydroxytyrosol, had minimum inhibitory concentrations of 125 and 1.90 µg/ml against *Vibrio cholerae*.

Conclusions

In the study performed, antibacterial activity of *Juglans regia* (walnut) and *Olea europaea* (29) oil was investigated against several bacteria affecting gut microbiome using disc diffusion method. From results it has been revealed that bacteria found in the are more susceptible to these. The bacterial inhibition of walnut oil and olive oil was mainly due to their chemical components, so further studies should be carried out to reveal other significant properties of these oils and control the major problem facing in the form of developing drug resistance by bacteria against antibiotics. These oils can be used for the development of novel medicines or in combination with the folk medicines to increase their effectiveness.

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