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Research Article

Antimicrobial potential of lemon and onion extracts against gram-positive and -negative bacteria

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Abstract: Antimicrobial potentials have been widely analyzed with different sources; however, plant-based antimicrobial compounds are greatly welcome due to their greener characteristics. This study revealed the importance of antimicrobial compounds from the herbal extracts of lemon and onion. The extracts were tested against gram-negative (*Escherichia coli*) and gram-positive (*Bacillus subtilis*) bacteria. Disc-diffusion and well-diffusion on an agar plate and tributary methods were followed to demonstrate the antimicrobial potentials of the above herbal extracts. Further, different volumes of ampicillin at the concentration of 1 mg/ml were used to compare the genuine bacterial inhibition (3 µL with 1.5 cm zone). Lemon behaved excellently in a way by displaying better bacterial inhibition against both *E. coli* (3 µL with 1.2 cm zone) and *B. subtilis* (3 µL with 0.6 cm zone), whereas onion extract was not at the level of lemon extract; however, it still displayed a good inhibition. The turbidity assay confirms the inhibition efficiency of lemon and onion against both *E. coli* and *B. subtilis.* In the liquid medium lemon shows higher inhibition (2 & 3 folds) on bacteria than that of ampicillin and onion. Cell count and UV-vis spectroscopy analysis at 600 nm also conform to the efficacy of lemon inhibition against *E. coli* and *B. subtilis.* This experiment confirms that lemon extract is an excellent and better substitute for commercially available ampicillin for bacterial inhibition.

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1. INTRODUCTION

Phytochemicals are compounds originally from plants, predominantly generated as secondary metabolites (Idehen *et al*., 2017; Choudhari *et al*., 2020). The secondary metabolites have been identified as terpenes, phenolic, and nitrogen compounds. For a long period, plant-based extracts were used to produce drugs against a range of diseases, called "traditional medicine". Most of the traditional medicines are considered to be "broad-spectrum" and found to have antimicrobial activities against a wide range of strains (Jamshidi-Kia *et al*., 2018; Osungunna 2020; Vaou *et al*., 2021). However, microbial disease transmission, especially bacteria show the genetic potential to acquire and transmit resistance to drugs. Identifying the natural compounds has antimicrobial activity as therapeutic agents are mandatory for medicinal purposes. The antimicrobial activity of natural products is beneficial, and they do not show side

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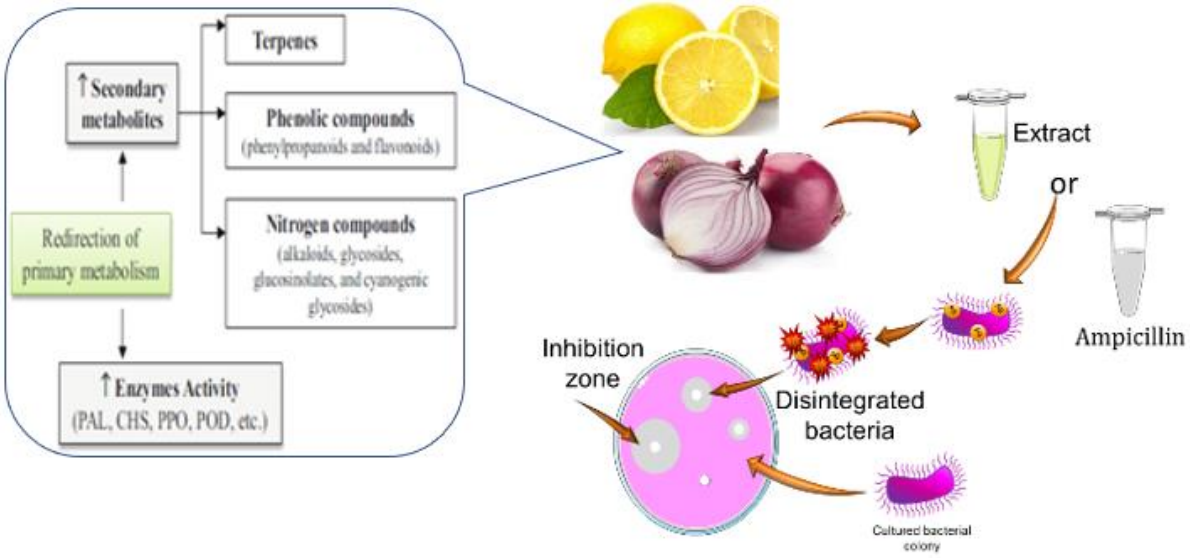
effects (Anyanwu & Okoye 2017). Among different plant species, citrus fruits are proven to have antifungal, antibacterial, antiviral, and anticancer activities (Kadhim Hindi & Ghani Chabuck 2013; Oikeh *et al*., 2016). Lemon (*Citrus limon*) is a commonly available inexpensive fruit that belongs to the Rutaceae family, popular for its medicinal and culinary uses. Lemon contains 5% of citric acid, and exhibits a higher level of vitamin C. Similarly, Onion (*Allium cepa*) is the oldest plant, containing minerals and vitamins. It is mainly used for culinary as well as medicinal purposes (Houba & Adam 1964; Ghaffariyan *et al*., 2012; Safaei-Chaeikar & Rahimi 2017). In this research, the antimicrobial activity of lemon and onion extracts was compared with the commercial antibiotic Ampicillin against bacterial pathogens. Ampicillin is the penicillin type of antibiotic and is used to treat several diseases caused by bacterial infections, including gonorrhoea, pneumonia, and salmonella. Even though it is effective against bacteria, it causes the side effects such as rash, fever, hives, vomiting, anaemia, nausea, headache, high count of white blood cells, and allergic reaction. So, it is mandatory to identify a suitable substitute for ampicillin with natural products to avoid the side effect. Herein, to reveal the plant-based antimicrobial compounds, lemon and onion extracts are tested against gram-negative and -positive bacterial species (Figure 1).

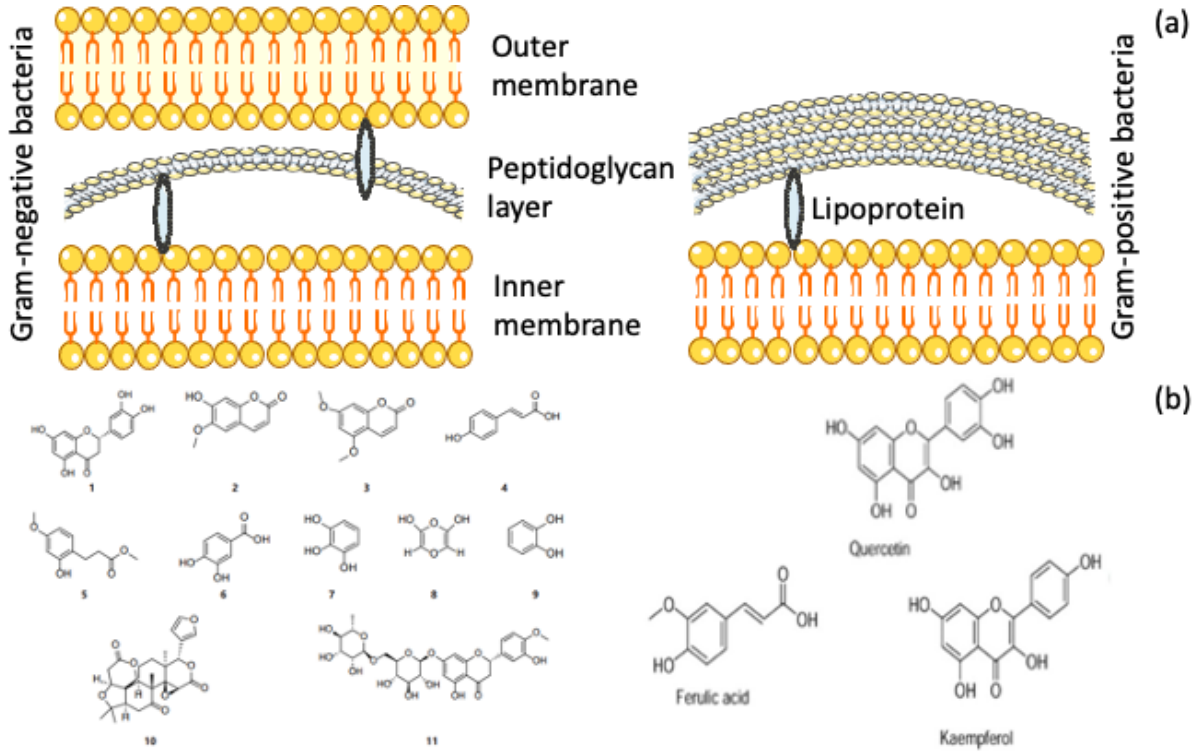
Figure 1. Primary metabolism of the plant. It results in increased synthesis of secondary metabolites. The secondary metabolites are metabolically classified into three main groups, terpenes, phenolic compounds, and nitrogen compounds. The steps involved in extraction and the mode of inhibition are displayed.



Two bacterial species namely *Escherichia coli* (*E. coli)* and *Bacillus subtilis (B. subtilis)* were evaluated for antimicrobial activity. These bacterial species belong to the class gram-negative and gram-positive bacteria, respectively. This discrimination is based on the gram-stain retaining capability of these species. Usually, gram-negative bacteria have a thin peptide-glycan layer and wash out the stain during the test. Whereas gram-positive bacteria have a thick peptidoglycan layer and retain the stain on the cell wall even after severe washing during the staining procedure (Figure 2a). The primary issue of microbial species is resistance to antimicrobial drugs, and it is considered one of the current challenges faced for long. However, researchers are continuously working on plant-based antimicrobial compounds as they are attractive and very often devoid of the side effects found to associate with synthetic antimicrobial compounds (Figure 2b). For the current study, two commonly occurring bacterial species (*E. coli* and *B. subtilis*] were considered to test the antimicrobial compounds from the edible plant sources. Strains of *E. coli* cause the illness of fever, diarrhea, vomiting, and

abdominal pain called an “opportunistic pathogen”. *B. subtilis* is widely spread in the open environment and causes food poison, called a “food pathogen”. Food-borne illnesses are an increasing global public health concern that necessitates more effective preservation techniques. Citric acid, ascorbic acid, minerals, flavonoids, and essential oils are just a few of the bioactive substances included in lemons. Similarly, the onion plant has a higher potential for antimicrobial compounds. To get more insight, the above bacterial survivals were analyzed by natural products of lemon and onion and compared with the established antibiotic “Ampicillin”.

Figure 2. Secondary metabolites and membrane structures. (a) Structure of the phenolic compounds in lemon. Eriodictyol (1), scopoletin (2), citropten (3), p-coumaric acid (4), 2-hydroxy4-methoxy benzene-propanoic acid methyl ester (5), protocatechuic acid (6), pyrogallol (7), diglycolic anhydride (8), catechol (9), limonin (10), hesperidin (11). (b) The difference in cell wall pattern from the bacteria. Gram-negative bacteria have thin peptidoglycan and gram-positive bacteria have thick peptidoglycan.



1.1. Mechanism of Antimicrobial Activity of Plant Extract Against Bacteria

Bioactive compounds such phenolics, flavonoids, ascorbic acid, vitamins, and essential oils are known to be present in citrus fruits. These compounds are believed to be the cause of a number of health advantages, including antibacterial, anti-inflammatory, anticancer, and antioxidant effects. The ability of phenolic compounds to act as electron donors in free radical reactions is commonly associated with antioxidant activity (Irkin *et al*., 2015; Hojjati & Barzegar, 2018). The main phenolic compounds of lemon are eriodictyol, scopolamine, citropten, protocatechuic acid, catechol, limonin, hesperidin, and diglycolic anhydride. Due to the antioxidant activity of phenolic compounds, lemon has been used for various purposes such as the formulation of healthy food, pharmaceutical and cosmetic products (M’hiri *et al*., 2017; Saifullah *et al*., 2019; Shaygannia *et al*., 2021). Figure 2b shows the structure of the phenolic compounds found in lemon. These phenolic compounds have the propionic side chain, which changes its behaviour as less polar than the protocatechuic acid (hydroxybenzoic acid). Considering the cell membrane permeability, caffeic acid is less polar, exhibits lipophilicity, and interferes with the permeability. Andrade *et al*., (2014) have proved that presence of α-tocopherol, lipophilic

compound damages the phospholipid and proteins in the membrane and affects these molecules by playing a pivotal role to increase membrane permeability. The catechin research has also proven that the number of alkyl chain carbons increases the antibacterial properties of these substances. This property facilitates the transport through the cell membrane and is related to the stronger antibacterial potential (Kępa *et al*., 2018).

Similarly, onions also contain high levels of phenolic compounds. The major phenolic compounds in onions are ferulic acid, gallic acid, kaempferol, quercetin, and chlorogenic acid (Figure 2b). Among these, gallic acid is at a higher rate and quercetin plays a major role with functional benefits such as anticancer, antivirus, antihistamine, and anti-inflammatory (Liguori *et al*., 2017). In addition, as stated above thicknesses in the bacterial membrane influences the penetration of these compounds, ultimately influencing the antibacterial action. It is hard to pinpoint the exact mechanism of molecular diffusion in the membrane, however, it is commonly agreed that the compounds penetrate through the periplasmic space of the cell wall and move into the cytoplasm, ultimately causing "pits" formation to the inner portion of the cell membrane. With this action, there is an enhancement in the cell permeability and causes more to be diffused, leading to the malfunction of the organism (Ramanathan & Gopinath 2017).

This research is aimed to compare the antimicrobial activity of extracts from lemon and onion with the commercial antibiotic "Ampicillin", to be tested against the bacterial strains *E. coli* and *B. subtilis.* Since the phenolic compounds and other secondary metabolites from the plant extract show excellent antimicrobial activity, we hypothesized to obtain good antimicrobial activities from the edible plants, lemon, and onion. It is expected that the experiments with both lemon and onion show a greater antimicrobial activity against the bacterial species and be better/comparable to ampicillin.

2. MATERIAL and METHODS

Agar, sterile water (H_2O), Ethanol, and Ampicillin were obtained from Sigma-Aldrich, USA. Hemocytometer (Thermo Fisher Scientific, USA) was used to count the bacteria. Lemon juice was prepared by directly squeezing using the bought fresh lemon from the local market (Malaysia). Onions were washed thoroughly with water and ethanol. And then juice was extracted from the juicer.

2.1. Hemacytometer: Bacterial Cell Counting

Cells are suspended in fluid and a small volume (~10 µL) of the fluid was placed into a special chamber. The hemacytometer was initially cleaned thoroughly using ethanol. The middle chamber with grid was loaded with 10 µL of the overnight culture and covered by the cover glass. The above set-up was placed under the microscope and counted with 5 different squares (with 16 internal squares). The measured counts were averaged for further experiments. The extraction procedure with lemon (*Citrus limon*) and onion (*Allium cepa*) was performed using mortar and pestle. The collected lemon and onion were washed thoroughly, and the pieces of sample were wetted during the grinding process.

2.2. Preparation of Agar-Nutrient Plates

The test microorganisms were cultured on nutrient agar (*Escherichia coli* and *Bacillus subtilis*). About 500 mL of water was added to 7 g of powdered agar-nutrient growth media, which were then autoclaved at 121 °C (15 min). To prevent the agar from setting, autoclaved media was cooled to 60 °C and aseptically placed into 20 cm Petri plates while maintaining sterility. Until they were utilized again, the unused plates were stored at 4°C.

2.3. Disc Diffusion Assay

Active cultures were prepared by transferring a single colony into the autoclaved culture media (5 ml of nutrient broth) and incubated (at 37 °C) for 24 h. The desired cultured bacterial count (10^6 cells) was spread independently on the agar plate. To measure the minimum inhibitory, different dilutions of lemon (as extracted) or onion (as extracted), or ampicillin (from 1 mg/mL) were wetted (with uniform final volume adjusted by the sterile water) a 5 mm-diameter paper disc that was placed on a plate of bacteria-containing agar and left to incubate there overnight. The inhibition areas formed were measured using the conventional ruler.

2.4. Well-Diffusion Assay

Active cultures were prepared by transferring a single colony into the autoclaved culture media (5 ml of nutrient broth) and incubated (at 37 °C) for 24 h. The desired cultured bacterial count (10^6 cells) was spread independently on the agar plate. Using the sterile cork-borer the uniform wells were made and poured with different dilutions of lemon (100% as extracted) or onion (100% as extracted) or ampicillin (from 1 mg/mL) and the uniform final volume was adjusted by the sterile water, then incubated overnight. The inhibition areas formed were measured using the conventional ruler.

2.5. Inhibitory Effect-Dose Dependent Analysis: Turbidity Assay

Active cultures were prepared by transferring a single colony into the autoclaved culture media (5 ml of nutrient broth) and incubated (at 37 °C) for 24 h. The desired cultured bacterial count (10^6 cells) was re-inoculated into each sterile culture tube containing 3 ml of culture medium. To the different tubes, varied amounts of lemon/onion extracts were added with equal final volumes by adjusting with sterile water. Kept all the culture tubes at 37° C incubator shaker overnight. The next day the cultures were measured for bacterial density using a UV-Visible spectrophotometer at 600 nm.

3. RESULTS

In this study, the extracts from edible parts of the plants (lemon and onion) were collected and tested for their antibacterial activities against *E. coli* and *B. subtilis*, and ampicillin was tested as a positive control. The experiment is designed to check the antimicrobial potential using the cultures in a liquid medium and on a solid agar surface. It is expected that depending on the diffusion and conjugation of the inhibitory compounds from the lemon/onion to the microbial cell wall, and the result will vary. Further, the antibacterial potential will differ from species to species. To make clear on this the selected test bacterial species are from gram-negative and -positive classes, originally categorized based on the gram-staining. Both gram-negative and -positive strains have different thicknesses of the peptidoglycan layers thin and thicker, respectively. This will make differences in the antibacterial activities due to dissimilar rates of penetration. In addition, the compounds from lemon and onion will display differences in inhibition due to the variations among the secondary metabolites extracted from the lemon and anion. To obtain solid results, both disc- and well-diffusion methods were followed and compared. Using a similar number of cells with the aid of a hemacytometer, different experiments were performed, and they yielded a higher confident output.

3.1. Diffusion Assay for Identifying the Antimicrobial Activity of Lemon and Onion Extract on *E. coli* and *B. subtilis*

Figure 3 shows the disc diffusion and well diffusion assay for Ampicillin, lemon, and onion against *E. coli*. As shown in Figure 3a, four different volumes (3.125, 6.25, 12.5, and 25 µL) with fixed concentration of Ampicillin, lemon, and onion extracts were tested against *E. coli*. It was noted that with increasing the volumes of Ampicillin, lemon, and onion, the diffusion was increased. At 3.125 µL, Ampicillin shows the highest diffusion, with increasing the

concentration to 6.25 µL, onion and lemon show the similar levels of diffusion (Table 1). In the good diffusion assay, lemon is more predominant than ampicillin and onion. Onion shows less diffusion than others. In the case of lemon, all four concentrations showed the highest rate of diffusion (Figure 3b). The diffusion rate of onion and lemon was close to ampicillin and in particular, lemon is closer to commercial Ampicillin (Figure 4a). It was noted that lemon extract works better than ampicillin and onion against *E. coli* (Figure 4a, b, Table 2).

Figure 3. (a) Results obtained by the disc-diffusion assay. (b) Results obtained by the disc-diffusion assay. Results for all three test samples were displayed against *E. coli*.

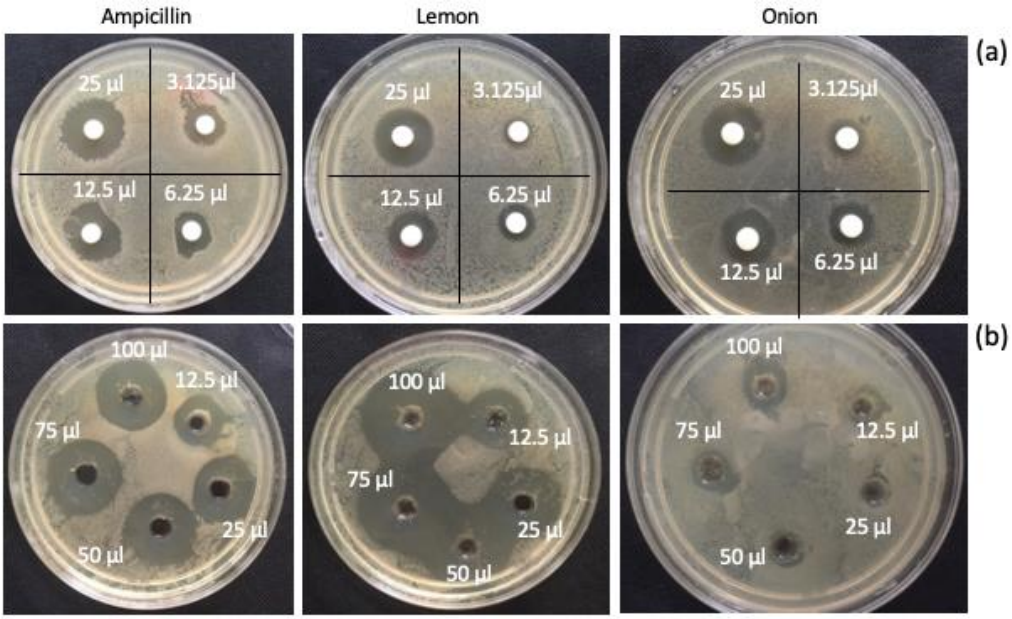


Figure 4. (a) Results obtained by the disc-diffusion assay. (b) Results obtained by the disc-diffusion assay. Results for all three test samples were displayed against *B. subtilis*.

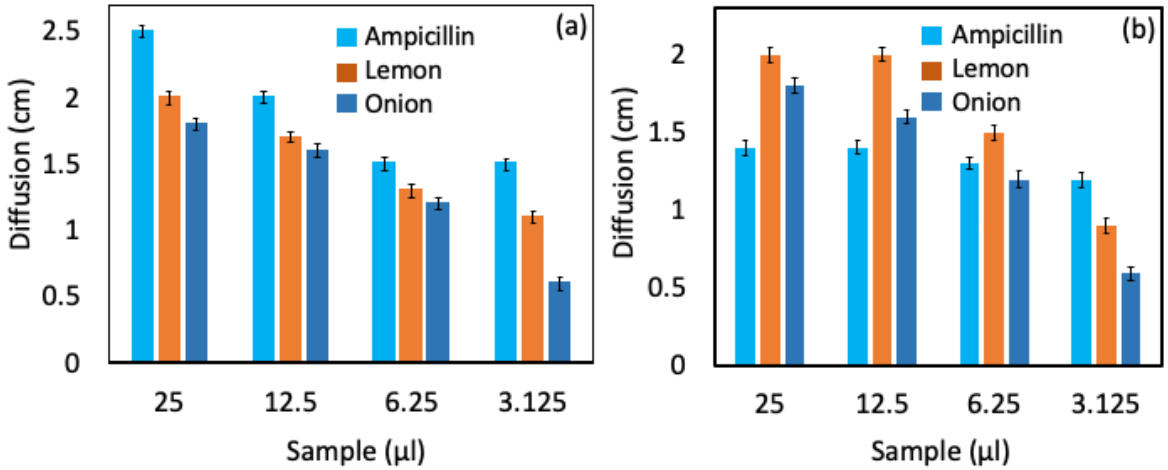


Table 1. Qualitative and quantitative assessments on disc diffusion assay.

Sample	Qualitative data		Quantitative data	
	E. coli	*B. subtilis*	*E. coli*	*B. subtilis*
Ampicillin	Smaller diffusion at 3 and 6 µL, larger diffusion at 12 and 25 µL	Smaller diffusion at 3 and 6 µL, larger diffusion at 12 and 25 µL	3 µL-1.5 cm 6 µL -1.5 cm 12 µL-2 cm 25 µL-2.5 cm	3 µL-1.2 cm 6 µL -1.3 cm 12 µL-1.4 cm 25 µL-1.4 cm
Lemon	With increasing the concentration, gradual increments in the inhibition	With increasing the concentration, gradual increments in the inhibition	3 µL-1.2 cm 6 µL -1.3 cm 12 µL-1.4 cm 25 µL-1.4 cm	3 µL-0.9 cm 6 µL -1.5 cm 12 µL-2 cm 25 µL-2.1 cm
Onion	With increasing the concentration, gradual increments in the inhibition	Diffusion depends on the concentration, but not clear enough.	3 µL-0.6 cm 6 µL -1.2 cm 12 µL-1.6 cm 25 µL-1.8 cm	3 µL-0.5 cm 6 µL -1.2 cm 12 µL-1.6 cm 25 µL-1.8 cm

Table 2. Qualitative and quantitative assessments of well diffusion assay.

	Qualitative data		Quantitative data	
	E. coli	*B. subtilis*	*E. coli*	*B. subtilis*
Ampicillin	With increasing the concentration, gradual increments in the inhibition	With increasing the concentration, gradual increments in the inhibition	12 µL-1.6 cm 25 µL -2 cm 50 µL-2.3 cm 75 µL-2.3 cm 75 µL-2.4 cm	12 µL-1.5 cm 25 µL -1.7 cm 50 µL-2 cm 75 µL-2 cm 75 µL-2 cm
Lemon	With increasing the concentration, gradual increments in the inhibition	With increasing the concentration, gradual increments in the inhibition	12 µL-1.7 cm 25 µL -2 cm 50 µL-2.7 cm 75 µL-2.7 cm 75 µL-3 cm	12 µL-2 cm 25 µL -2.5 cm 50 µL-2.8 cm 75 µL-3 cm 75 µL-3.4 cm
Onion	Small diffusion was noticed	Small diffusion was noticed	12 µL-1.1 cm 25 µL -1.1 cm 50 µL-1.1 cm 75 µL-1.2 cm 75 µL-1.3 cm	12 µL-0.8 cm 25 µL -0.8 cm 50 µL-1 cm 75 µL-1.2 cm 75 µL-1.3 cm

Similar experiments were conducted with the gram-positive bacteria *B. subtilis***.** Figure 5 shows the disc diffusion and well diffusion assays for Ampicillin, lemon, and onion against *B. subtilis***.** As shown in Figure 5a, b, four different volumes (3.125, 6.25, 12.5, and 25 µL) with fixed concentrations of Ampicillin, lemon, and onion were tested against *B. subtilis***.** In this case, lemon works well in both disc diffusion and well diffusion assay for all concentrations against *B. subtilis***.** It was visibly observed with lemon, the diffusion was spread widely in all the areas of the plate and is greater than the plate with Ampicillin. The length of diffusion was higher with lemon followed by ampicillin and onion in both disc diffusion and well diffusion assay (Figure 6a, b). From this result, it was concluded that lemon and onion work well against *E. coli* and *B. subtilis*. In particular, lemon works better than commercially available Ampicillin.

Figure 5. Disc diffusion assay. (a) *E. coli*; (b) *B. subtilis*. Inhibition area (cm) by ampicillin, lemon, and onion are shown. Data are averaged with triplicates and indicated by error values.

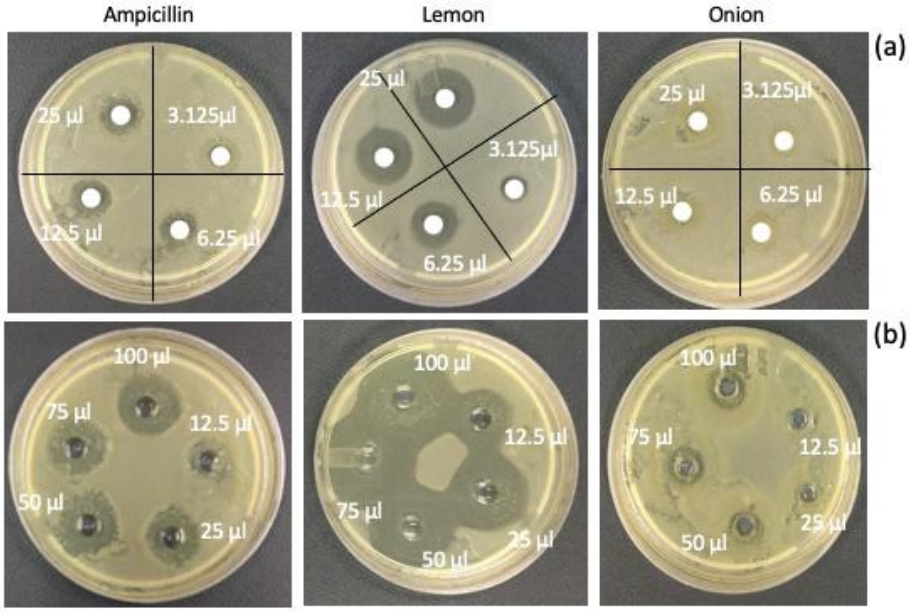
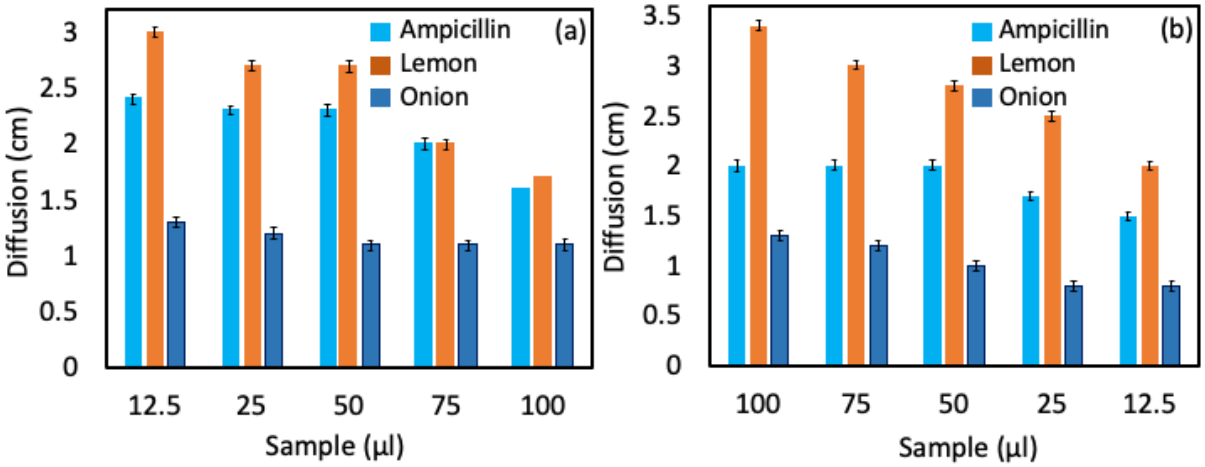


Figure 6. Well diffusion assay. (a) *E. coli*; (b) *B. subtilis*. Inhibition area (cm) by ampicillin, lemon, and onion are shown. Data are averaged with triplicates and indicated by error values.



3.2. Turbidity Assay for Identifying the Antimicrobial Activity of Lemon and Onion Extract on *E. coli and B. subtilis*

Since lemon and onion suppress the growth of *E. coli* and *B. subtilis*, further results were confirmed with the liquid medium by turbidity assay. In the liquid medium, the bacteria were grown and treated with lemon, onion, and ampicillin, and the results were compared. As shown in Figure 7, lemon shows an equal level of inhibition with commercially available ampicillin in both *E. coli* and *B. subtilis*. Onion inhibits the growth of bacteria at higher concentrations. The samples are further analyzed with UV-Vis spectroscopy. The optical density of the solution was measured. The liquid treated with lemon and ampicillin shows a lower O.D. compared with inion (Table 3). Lemon shows lower OD at higher concentrations compared with ampicillin. This result confirmed that lemon works well than ampicillin in a liquid medium compare with a solid place. For further confirmation the changes were validated by cell counting and measurement on biomass.

Figure 7. Turbidity assay for qualitative assessment. +++: Good growth; ++ Moderate growth; + Less growth; - Not significant.

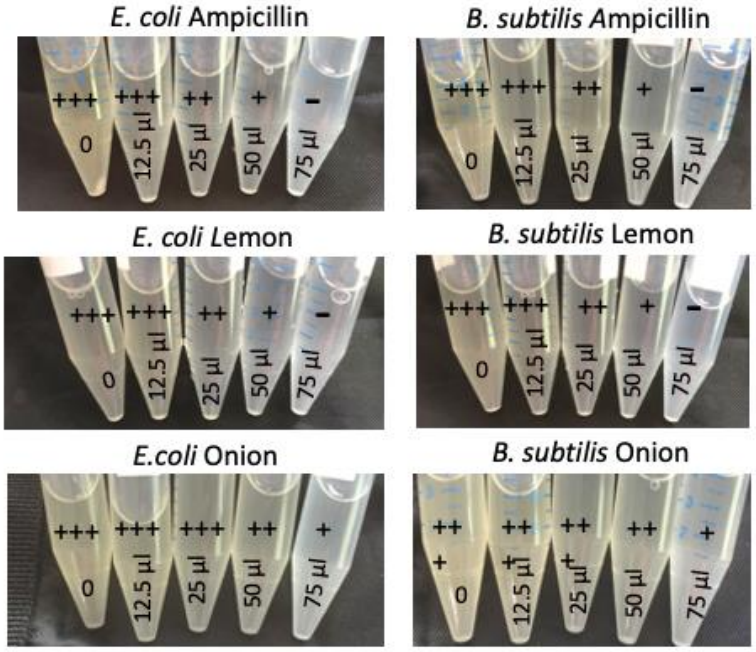


Table 3. Inhibitory Effect-dose dependent analysis (after 24 hrs; Turbidity).

Sample volume	Ampicillin (O.D)		Lemon (O.D)		Onion (O.D)	
	E.coli	*B. subtilis*	*E. coli*	*B. subtilis*	*E. coli*	*B. subtilis*
0	0.9	0.8	0.9	0.8	0.9	0.8
12.5 µL	0.85	0.74	0.78	0.72	0.86	0.77
25 µL	0.72	0.69	0.62	0.68	0.79	0.7
50 µL	0.61	0.57	0.54	0.59	0.71	0.59
75 µL	0.4	0.5	0.33	0.48	0.65	0.53
100 µL	0.2	0.4	0.11	0.3	0.3	0.43

3.3. Cell Count and Biomass of the Bacteria in the Liquid Medium

For further confirmation of the effect of lemon and onion effect against the bacteria, cell count and biomass were calculated. The following are the basic calculation made with the hemacytometer measurements.

The number of cells/ cubic mm = Number of cells counted/ mm^2 X dilution X 10

The number of cells/mm =Number of cells counted / mm^2 X dilution X 10 000

As shown in Figure 8a, cell count was decreased when increasing the concentration of lemon, onion, and ampicillin. Lemon shows the lowest level of cell count compared with ampicillin and lemon (Tables 4 & 5). Similarly, the Inhibitory effect-bacterial biomass was calculated for further confirmation. The cultures were filtered on a pre-weighed Whatman filter paper. The collected biomass was dried in an oven at 80°C. After drying the final weight was calculated by subtracting the initial weight of the filter. Figure 8b shows the biomass of the bacteria from the liquid treated with lemon, onion, and ampicillin. In both of the bacteria, lemon shows a lower mass of bacteria, which confirms the strong inhibition of lemon against bacteria compared with ampicillin and lemon.

Figure 8. Quantitative analysis with bacterial growth. Cell count (a) and Biomass (b) analyses are shown.

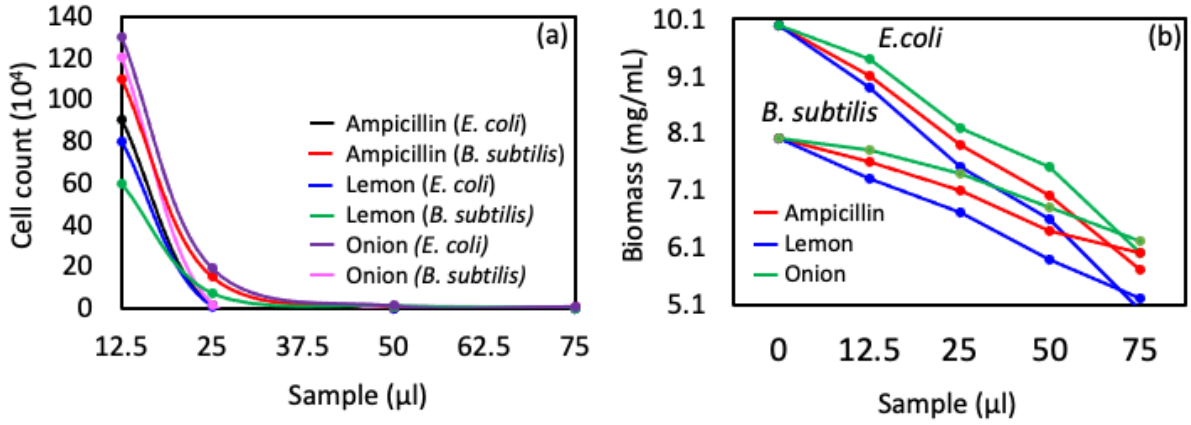


Table 4. Biomass & Cell count-Dose dependent analysis (after 24 hrs: *E. coli*).

Sample volume (µL)	Ampicillin		Lemon		Onion	
	Biomass (mg/ml)	Cell count	Biomass (mg/ml)	Cell count	Biomass (mg/ml)	Cell count
0	10	18 x 10^6	10	18 x 10^6	10	18 x 10^6
12.5	9.1	0.9 x 10^5	8.9	0.8 x 10^5	9.4	12 x 10^5
25	7.9	1.1 x 10^4	7.5	0.91 x 10^4	8.2	1.7 x 10^4
50	7.0	0.2 x 10^4	6.6	0.6 x 10^3	7.5	0.8 x 10^4
75	5.7	0.7 x 10^3	5.0	0.1 x 10^3	6.0	0.1 x 10^4

Table 5. Biomass & Cell Count-Dose dependent analysis (after 24 hrs: *B. subtilis*).

Sample volume (µL)	Ampicillin		Lemon		Onion	
	Biomass (mg/ml)	Cell count	Biomass (mg/ml)	Cell count	Biomass (mg/ml)	Cell count
0	8	16 x 10^5	8	16 x 10^5	8	16 x 10^5
12.5	7.6	11 x 10^5	7.3	6 x 10^5	7.8	13 x 10^5
25	7.1	1.5x 10^5	6.7	1x 10^4	7.4	1.9x 10^5
50	6.4	1 x 10^4	5.9	0.9 x 10^3	6.8	1.2 x 10^4
75	6.0	0.5 x 10^4	5.2	0.4 x 10^3	6.2	0.9 x 10^4

4. CONCLUSION

Research in the medical field is necessary to improve human health and to increase human life span. Different research is going on about threatening diseases including the diseases caused by bacteria and viruses. There are different ways to treat microbial infections; however, the currently available medicines cause side effects. Natural medicine is better because it can avoid side effects; particularly herbal plants can cure microbial infections. Towards this direction, this study has been launched to make the expansion for the potential applications of the plant extracts to be used as antibacterial agents. For this analysis, two common edible plants, namely lemon and onion have been chosen for the gram- and gram-positive bacteria, namely *E. coli* and *B. subtilis*, respectively. The following conclusions have been made based on the obtained results. With the disc-diffusion assay the commercial antibiotic, ampicillin, lemon, and onion can inhibit the growth of *E. coli* and *B. subtilis*. In the case of *E. coli*, ampicillin shows the highest inhibition followed by lemon and onion. Interestingly in a well diffusion assay lemon has higher antibacterial activity against *E. coli* and *B. subtilis*. Followed by lemon extract, ampicillin displays better antibacterial activity against *B. subtilis*, whereas onion shows the least activity. The biomass obtained from the liquid medium inhibition assays support the results by showing a reduction in the biomass compared to the control test in the absence of additional compounds. Cell counts performed with the same assays show the decrement in the cell count with increasing the plant extract or ampicillin concentration. Considering the limitation with the above methods, inclusion of filtration step with lemon or onion extract makes changes in the antimicrobial activity due to the attachment of important compounds on the filters. In addition, when go for a large scale needs a fine optimization, and the above methods work also well with different sources of plant material.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Authorship Contribution Statement

Nagomi Gopinath: Investigation, Resources, Visualization, Software, Formal Analysis, Methodology, Supervision, and Validation. and Writing -original draft.

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