

# Innovations

## Evaluation of Antimicrobial Activity of Plant Extracts Against *Escherichia coli* and *Aspergillus Sp.*

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**Abstract:** *In the present study, a total of 22 plant extracts was screened for their antimicrobial activity against E. coli and Aspergillus Sp. by agar well diffusion method. The highest antibacterial activity was shown by the Syzygium leave aqueous extract, followed by the Zingiber root ethanolic, and Azadirachta leave ethanolic extracts against E. coli with zone of inhibition 20mm, 18mm and 18mm respectively. Murraya leave ethanolic extract showed inhibitory activity with the zone of inhibition of 11mm against E. coli. The zone of inhibition of ethanol extract of Piper leaves has shown the zone of inhibition of (30mm) followed by Curcuma root ethanolic extract (15mm) against Aspergillus Sp. The maximum activity was found in Ketoconazole with the zone of inhibition of 15mm followed by clotrimazole (10mm) and Itraconazole (8mm). Aspergillus Sp. was found to be resistant to Nystatin, Amphotericin B and Fluconazole. E. coli was found to be sensitive to Gentamicin and clindamycin and it was resistant to Ampicillin, Cephalothin, Chloramphenicol, Oxacillin, Vancomycin and Erythromycin. The minimum inhibitory concentration of the aqueous extract of Syzygium leave extract against E. coli was 2.5%. The minimum inhibitory concentration of the ethanol extract of Piper fruit against Aspergillus Sp. was 10% (w/v). Phytochemical analysis of Piper fruit ethanolic extract showed the presence of alkaloids, flavonoids, phenolic compounds, proteins and amino acids, carbohydrates, and absence of terpenoids. It can be suggested from the present study that Piper ethanolic extract may be used as natural antifungal agent against the infection caused by Aspergillus Sp. and Syzygium leaves aqueous extract against E. coli.*

**Keywords:** *Antimicrobial activity, agar well diffusion method, Aspergillus and E. coli.*

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## 1.0 Introduction

The term human microbiota refers to all microbiota that lives on or in human tissues and biofluids, as well as the gastrointestinal system and skin. The human body contains a variety of microbiota, including bacteria and fungi (Alarcon et al., 2017). Bacterium like *Escherichia coli* lives in the human gastrointestinal tract. *E. coli* is a facultative anaerobic, rod-shaped, gram negative bacteria that lives in the human gastrointestinal tract and harmless and usually lives in the intestine of healthy humans and animals. A common Gram-negative bacteria found in both teaching and research labs is *E. coli* (Tuttle et al, 2021).

Similarly, one of the most abundant group of fungi in the world is represented by the genus *Aspergillus* Spp. In healthy or normal hosts, aspergillosis is an airborne fungus that does not cause illness; but, in immunocompromised hosts, it can lead to a serious infection. (ThwePhyoKanNyunt et al., 2020). Concern over *Aspergillus* species developing resistance to the azole antifungal voriconazole, posaconazole, and itraconazole antifungal. This is particularly concerning for *A. fumigatus*, as acquired resistance has been reported in both azole native persons and patients with invasive illness cause by this species that were exposed to these compounds (Wiederhold et al., 2015). People who have diabetes mellitus are more vulnerable to non-standard infections and have a weakened immune system, which increases risk (Li et al., 2024). Triazole resistance is an increasing concern in the opportunistic mold *Aspergillus fumigatus* (Sharpe et al., 2018).

*Escherichia coli* is a fecal bacteria that lives in the natural environment secondary habitats and the intestines of endotherms primary habitat (Touchon et al., 2020). *E. coli*, Gram negative bacteria and yeast against which the antimicrobial activity of five different solvents—methanol, ethanol, distilled water, chloroform, and petroleum ether—was evaluated using the agar well diffusion method (Sharma and Pundir 2018).

The medicinal plants can be used as a alternative to these antibiotics, the medicinal plants were being used from the ancient times to treat diseases like fever cough cold, cancer, diabetes and fungal infections etc. these medicinal plants can be used to treat several diseases including bacterial and fungal infections by using agar well diffusion method we can easily visualise the effects of the medicinal plants on the fungi and bacteria like *Escherichia coli*.

Phytochemical analysis of the plant can help to determine the compounds such as secondary metabolites macro and micronutrients including phytochemicals such as alkaloids, flavonoids, Tannins present within the plants. All parts of the plant, including the roots, stem, leaves, flowers, fruits, and seeds, are sources of these

phytochemicals. These phytochemicals are occasionally employed as such and occasionally serve as the starting point for a wide range of other substances with significant medical applications (Balamurugan et al., 2019).

Antimicrobial Testing aims to ensure susceptibility to preferred medications for specific diseases and identify potential treatment resistance in common organisms. Antimicrobial susceptibility testing is done to help ensure that the right antimicrobial therapy is used to maximize treatment outcomes. It does this by providing in vitro data. The gradient technique known as Etest integrates the concepts of the agar dilution and disk diffusion techniques (Wanger et al., 2021).

One of the most reliable ways to guarantee that everyone on the planet has access to health care through techniques that are socially acceptable, secure, and financially viable is through traditional medicine. Medicinal plants do have future perspective in the field of medication and they can be used as an alternative to pharmaceutically available antibiotics. Keeping in view the above justification present study is to screen plant extracts against *E. coli* and *Aspergillus* Sp. and phytochemical analysis of best antimicrobial plant extract.

## 2.0 Materials and Methods

### 2.1 Procurement of bacterial cultures

The test organisms: *Escherichia coli* and *Aspergillus* Sp, were procured from Department of Biosciences, Chandigarh University, Mohali, Punjab and maintained the cultures at 4° C and sub-cultured on a regular basis.

### 2.2 Collection of plant and preparation of plant extracts

Collection of selected medicinal plants such as leaves of the *Nerium* Sp., *Lantana* Sp., *Murraya* Sp., *Syzygium* Sp., *Catharanthus* Sp., *Azadirachta* Sp., stem of the *Tinospora* Sp., root of the *Curcuma* Sp., *Zingiber* Sp., and flower bud of *Syzygium*Sp., and fruit of the *Piper* Sp., were screened for their antimicrobial activity.

The plants were Collected from different regions of Mandi,(Himachal Pradesh) and Mohali,( Punjab)India due to the different climatic conditions and soil composition the availability of the plants is in different regions. The collected plant parts were brought to the laboratory and washed it properly with tap water. Spread the plant parts on silver foil properly and placed the silver foil paper in the oven and dried in a controlled temperature 60°C for 12-24 hours. The dried parts were crushed into a fine powder by using a mortar and pestle ensuring homogeneity. 20% w/v of the plant extract was prepared in different solvents such as distilled water, ethanol and

petroleum ether. The powder was mixed with solvents and kept at room temperature for 24hrs. The extract was filtered using the Whatman filter paper and the filtered extracts were stored in the refrigerator for the further experiment (Enejiyon et al., 2020)

### **2.3 Antibacterial activity of the plant extracts by agar well diffusion method**

Antibacterial studies were tested using agar well diffusion method (Chavez et al., 2021). Sterile nutrient agar plates were prepared. 100µl of standardised bacterial and fungal suspensions ( $1.5 \times 10^6$  cells per ml) was spread on sterilized NA plates using sterile cotton swabs. 6mm wells were punched on agar plates using sterilized borer. 50-100 microliter of each plant extract was poured in the wells. Plates were incubated at 37°C for 24 hours for bacteria and 27°C for 3-4 days for fungus culture. Solvents were poured in wells used a negative control and antibiotics were used as positive control as shown in antibiotic susceptibility pattern of test microbes. Zone of inhibition was observed and diameters of ZOI were measured using transparent ruler meter rule in mm.

### **2.4 Antibiotic Susceptibility Pattern of Test microbial culture**

Antibiotic susceptibility pattern test microbes was done by using Kirby-Bauer disc diffusion method (Yao et al., 2021). In this method, 100 microliter of both the microbial suspensions was spread on sterilized NA and PDA plates using sterile cotton swabs. Antibiotic disc (HI Media, Mumbai) was placed on the surface of agar plates having seeded culture suspension plates. The plates were incubated at 37°C for 24 hours for bacterium and 27°C for 3-4 days for fungus culture and then ZOI was observed and diameter of ZOI was recorded in mm.

### **2.5 Minimum inhibitory concentration(MIC)of best plant extracts**

The minimum inhibitory concentration (MIC) is the highest dilution or the least concentration of an antimicrobial agent that will inhibit the growth or kill the microorganism. Macro dilution agar plate method was used to determine MIC of most potent plant extract. Different concentrations (20%-0.25% w/v) of best antimicrobial plant extract were prepared by two fold dilution. 6mm well were cut in the agar plates with sterile borer poured different concentration into the well with sterile tips. Then the plates were incubated at 37°C for 18-24 hours for bacterial and 27°C for 3-4 days for fungal growth. After incubation, the plates were observed for ZOI and determined the MIC against bacterial and fungal cultures (Pundir et al., 2010).

## 2.6 Phytochemical analysis

The phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, tannins, terpenoids were analysed by using the method given in (Sahrawat et al., 2018).

## 3.0 Results

### 3.1 Antibacterial activity of plant extracts

The antibacterial activity of the selected plants *Nerium* Sp., *Lantana* Sp., *Murraya* Sp., *Syzygium* Sp., *Catharanthus* Sp., *Tinospora* Sp., *Azadirachta* Sp., *Curcuma* Sp., *Syzygium* Sp., *Zingiber* Sp., *Piper* Sp., ethanolic, and distilled water extracts was screened against *E. coli* by agar well diffusion method. Out of 22 plant extracts the highest antibacterial activity was shown by the *Syzygium* leaf aqueous extract, followed by the *Zingiber* root ethanolic, and *Azadirachta* leaf ethanolic extracts against *E. coli* with zone of inhibition 20mm, 18mm and 18mm respectively. *Murraya* leaf ethanolic extract showed inhibitory activity with the zone of inhibition of 11mm against *E. coli*.

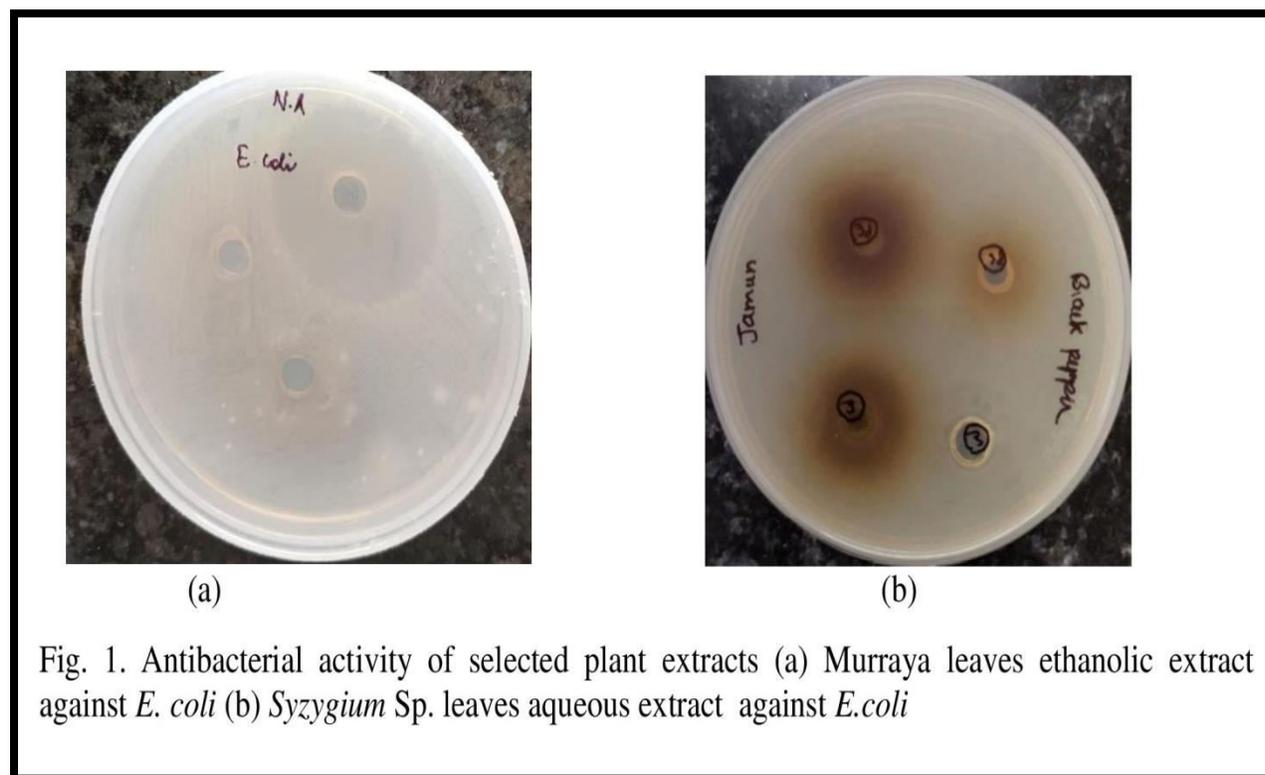


Fig. 1. Antibacterial activity of selected plant extracts (a) *Murraya* leaves ethanolic extract against *E. coli* (b) *Syzygium* Sp. leaves aqueous extract against *E. coli*

### 3.2 Antifungal activity of plant the selected plant extracts

By using the agar well diffusion method for the antifungal activity of the selected plants *Nerium Sp.*, *Lantana Sp.*, *Murraya Sp.*, *Syzygium Sp.*, *Catharanthus Sp.*, *Tinospora Sp.*, *Azadirachta Sp.*, *Curcuma Sp.*, *Syzygium Sp.*, *Zingiber Sp.* and *Piper Sp.* Among all of them the best antifungal activity against the *Aspergillus* was shown by *piperSp* fruit ethanolic extract. Maximum zone of inhibition was seen in *piper Sp.*, in the selected fungus. The zone of inhibition of ethanol extract of the *piper Sp.*, has shown the zone of inhibition of 30mm in *AspergillusSp.* followed by *curcumaSp.* with the zone of inhibition of 15mm in *Aspergillus Sp.* as shown in Table 1 and Fig 2.

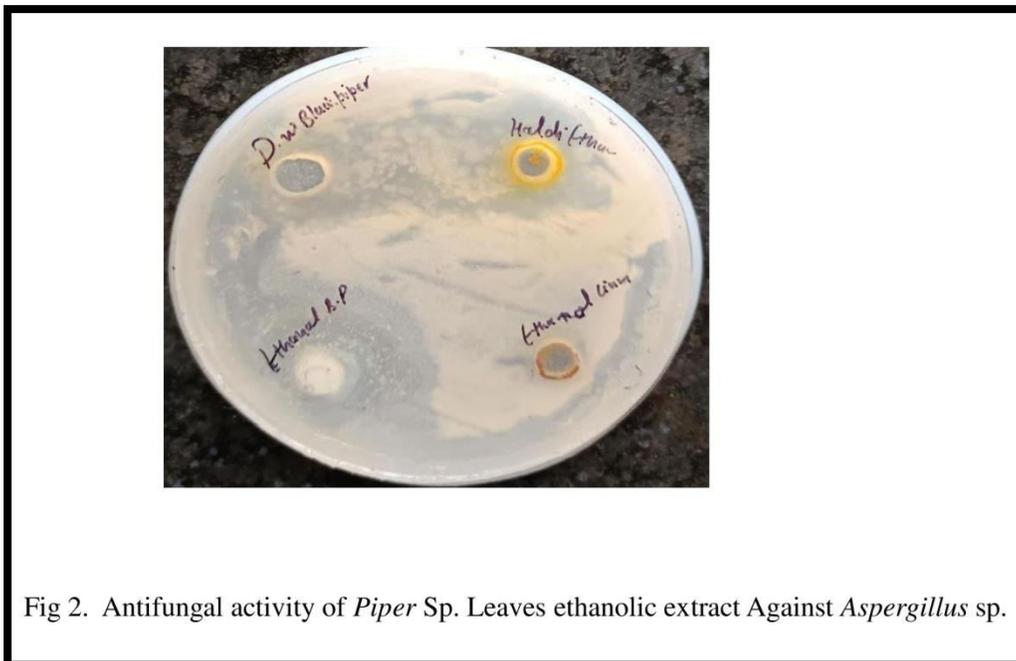


Fig 2. Antifungal activity of *Piper Sp.* Leaves ethanolic extract Against *Aspergillus sp.*

### 3.3 Antibiotic Susceptibility Pattern of *Aspergillus Sp.* and *E.coli*

The antifungal susceptibility test was done by antibiotic disc diffusion method. The antifungal activity was seen in different antibiotics such as clotrimazole, fluconazole, Ketoconazole, Itraconazole, Nystatin and Amphitericin B for *Aspergillus*. The maximum activity was found in Ketoconazole with the zone of inhibition of 15mm followed by clotrimazole (10mm) and Itraconazole (8mm). Nystatin, Amohitericin B and Fluconazole did not show any inhibitory activity against *Aspergillus Sp.* Similarly *E.coli* was found to be sensitive to Gentamicin and clindamycin shown in Fig 3. it was resistant to Ampicillin, Cephalothin, Chloramphenicol, Oxacillin, Vancomycin and Erythromycin (Table 2).

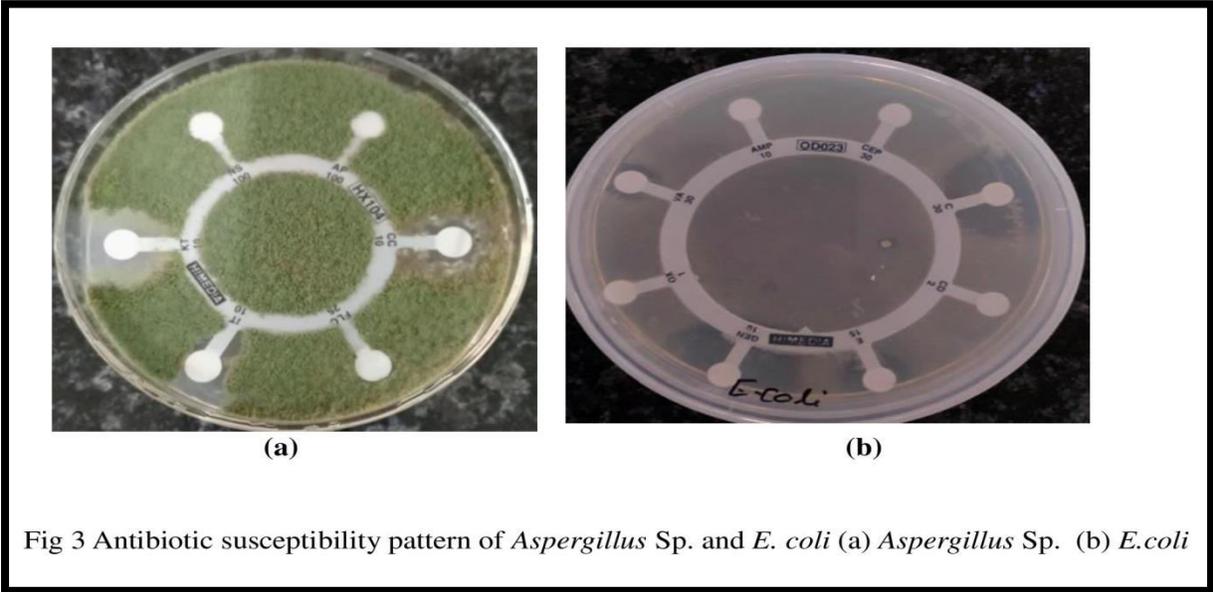


Fig 3 Antibiotic susceptibility pattern of *Aspergillus* Sp. and *E. coli* (a) *Aspergillus* Sp. (b) *E. coli*

Table2. Antibacterial susceptibility pattern of *E. coli* and *Aspergillus* Sp.

Antibiotics	Symbol	Concentration	Zone of inhibition in <i>E. coli</i>	Antibiotics	Symbol	Concentration	Zone of inhibition in <i>Aspergillus</i> Sp.
Ampicillin	AMP	10mcg	NA	Amohite ricin B	AP	100umits	NA
Cephalothin	CEP	30mcg	NA	Fluconazole	FIC	25mcg	NA
Chloramphenicol	C	30mcg	NA	Ketoconazole	KT	10mcg	15mm
Clindamycin	CD	2mcg	11mm	Itraconazole	IT	10mcg	8mm
Gentamicin	GEN	10mcg	15mm	Nystatin	NS	100 units	NA
Oxacillin	OX	1mcg	NA	Clotrimazole	CC	10mcg	10mm
Vancomycin	VA	30mcg	NA				
Erythromycin	E	15mcg	NA				

NA: No activity

### 3.4 Minimum inhibitory concentration of the best plant extracts (aqueous extract of *Syzygium* Sp. and ethanol extract of *Piper* Sp. fruit)

The minimum inhibitory concentration of the aqueous extract of *Syzygium* Sp. leave against *E. coli* was 2.5%. The minimum inhibitory concentration of the ethanol extract of *Piper* Sp. fruit against *Aspergillus* Sp. 10% as shown in Table 3.

Table 3. Minimum inhibitory concentration of *Piper* fruit ethanolic extract against *Aspergillus* Sp. and Aqueous extract of *Syzygium* leaves against *E. coli*

Plant extract	Concentration 20%	Concentration 10%	Concentration 5%	Concentration 2.5%	Concentration 1.25%	MIC of <i>Piper</i> Sp (W/V)
Ethanolic plant extract of <i>Piper</i> sp. Against <i>Aspergillus</i>	-	-	+	+	+	10% (w/v)
Ethanolic plant Aqueous extract of <i>Syzygium</i> sp. Against <i>E. coli</i>	-	-	-	-	+	2.5% (w/v)

### 3.5 Phytochemical analysis of *Piper* Sp. Fruit ethanolic extract

Phytochemical analysis of *Piper* fruit ethanolic extract showed the presence of alkaloids, flavonoids, phenolic compounds, proteins and amino acids, carbohydrates, Tannins and absence of terpenoids as shown in Table 4.

Table 4. Phytochemical analysis of ethanolic extract of *Piper* Sp.

Plant Used	Phytochemical Constituents						
	Alkaloids	Flavonoids	Phenolic compounds	Terpenoids	Proteins	Carbohydrates	Tannins
ethanolic extract of <i>Piper</i> Sp.	+	+	+	-	+	+	+

+: Presence; -: Absence

#### 4.0 Discussion

Using agar well diffusion method, the antibacterial activity of the selected plants *Nerium sp.*, *Lantana sp.*, *Murraya*, *Syzygium*, *Catharanthus*, *Tinospora*, *Azadirachta*, *Curcuma*, *syzygium*, *Zingiber*, *Piper species* ethanolic, and distilled water extracts. Out of 22 plant extracts, the highest antibacterial activity was shown by the *Syzygium* Sp. leave aqueous extract, followed by the *Zingiber* Sp. root ethanolic, and *Azadirachta* Sp. leave ethanolic extracts against *E. coli* with zone of inhibition 20mm, 18mm and 18mm respectively. *Murraya* leave ethanolic extract showed inhibitory activity with the zone of inhibition of 11mm against *E. coli*. According to the findings of the antibacterial test, the *Syzygium* sp. plant's extracts and leaf fractions can stop the growth of germs (Klaumegio et al., 2021). The biggest diameter inhibition zone measured at 15.03 mm was found in the results, which indicated that 50% of ginger extract had the best antibacterial action. We can infer from this work that red ginger extract possesses antibacterial properties that can inhibit the growth of *Escherichia coli* germs (Yang et al., 2022).

*Murraya koenigii* extracts have demonstrated antibacterial effects particularly on *E. coli* (Harbi et al., 2016). The minimum inhibitory concentration (MIC) of *A. indica* leaf extract was shown to be significantly lower in vitro (50 mg/ml) against *E. coli* than it was against *S. aureus* (100 mg/ml). The leaf extract of *A. indica* has a bacteriostatic activity against gram negative bacteria and a static effect against gram positive bacteria, as indicated by the minimum bactericidal concentration (MBC) of the extract (Muhammad et al, 2019) Other plants extracts were shown no as such activity maybe due the soil composition of the area from where plants were Collected or due to the different climatic conditions or different plant species etc. They have future aspects for further studies. The maximum activity was found in Ketoconazole with the zone of inhibition of 15mm followed by clotrimazole (10mm ) and Itraconazole (8mm). Nystatin, Amohitericin B and Fluconazole did not show any inhibitory activity against *Aspergillus* Sp. Triazole resistance is an increasing concern in the opportunistic mold *Aspergillus fumigates* *E. coli* was found to be sensitive to

Gentamicin and clindamycin and it was resistant to Ampicillin, Cephalothin, Chloramphenicol, Oxacillin, Vancomycin and Erythromycin. *E. coli* isolates were resistant to enrofloxacin, tetracycline, ampicillin, co-trimoxazole, gentamicin, and 139/165 (85%), 157/165 (95.4%), 154/165 (93.6%), 141/165 (86%), and 139/165 (82.7%), in that order (Elderdiri et al., 2022).

This antibiotics resistance against bacteria is a concern. The alternative to this maybe the medicinal plants parts which are being used for the ancient times. The minimum inhibitory concentration of the aqueous extract of *Syzygium* leave against *E. coli* was 2.5%. The minimum inhibitory concentration of the ethanol extract of *Piper* fruit against *Aspergillus* Sp. was 10% (w/v). With an inhibition zone width range of 22.1 mm-11.5 mm against Gram positive and 21.8 mm-12.4 mm against Gram negative bacteria, leaf extract clearly shows the greatest potency as an antibacterial. The extract's minimum inhibitory concentrations (MICs) were found. Gram negative bacteria had a MIC of 208 µg/ml while Gram positive bacteria had a MIC of 104 µg/ml. The study examined the synergistic effect of *S. cumini* leaf extract with six antibiotics against isolates with higher resistance, which seemed to enhance the antibiotic action (Jassim et al., 2024).

Phytochemical analysis of *Piper* Sp., fruit ethanolic extract showed the presence of alkaloids, flavonoids, phenolic compounds, proteins and amino acids, carbohydrates, Tannins are present in the plant and absence of terpenoids etc. The extracts included flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides, and tannins, according to preliminary phytochemical analyses. Because *Syzygium cumini* contains a high concentration of phytochemicals, it has remarkable therapeutic potential. The objective of the current study was to assess the anti-diabetic potential of the seeds of the *S. cumini* plant. Kaempferol and gallic acid, the two most abundant phytochemicals, were chosen for additional examination (Rashid et al., 2022).

## 5.0 Conclusion

Using agar well diffusion method, the antibacterial activity of the selected plants *Nerium* Sp., *Lantana* sp., *Murraya* Sp, *Syzygium* Sp, *Catharanthus* Sp, *Tinospora* Sp,, *Azadirachta* Sp, *Curcuma* Sp, *Syzygium* Sp, *Zingiber* Sp, *Piper* Sp, ethanolic, and distilled water extracts. Out of 22 plant extracts the highest antibacterial activity was shown by the *Syzygium* leave aqueous extract, followed by the *Zingiber* root ethanolic, and *Azadirachta* leave ethanolic extrats against *E. coli* with zone of inhibition 20mm, 18mm and 18mm respectively. *Murraya* leave ethanolic extract showed inhibitory activity with the zone of inhibition of 11mm against *E. coli*. The zone of inhibition of ethanol extract of *Piper* fruit has shown the zone of inhibition of (30mm) followed by *curcuma* root (15mm) against *Aspergillus* Sp. The maximum activity was found in

Ketoconazole with the zone of inhibition of 15mm followed by clotrimazole (10mm ) and Itraconazole (8mm). Nystatin, Amohitericin B and Fluconazole did not show any inhibitory activity against *Aspergillus* Sp. *E. coli* was found to be sensitive to Gentamicin and clindamycin and it was resistant to Ampicillin, Cephalothin, Chloramphenicol, Oxacillin, Vancomycin and Erythromycin. The minimum inhibitory concentration of the aqueous extract of *Syzygium* leave against *E. coli* was 2.5%. The minimum inhibitory concentration of the ethanol extract of *Piper* fruit against *Aspergillus* Sp. was 10% (w/v). Phytochemical analysis of *Piper* fruit ethanolic extract showed the presence of alkaloids, flavonoids, phenolic compounds, proteins and amino acids, carbohydrates, Tannins are present in the plant and absence of terpenoid. The purpose of the study was to determine how effective certain plant extracts were against selected bacteria and fungi. Here, the selected fungus is *Aspergillus* and the selected bacteria is *E. coli*. The selected herbal medicinal plants were being used from ancient times to treat the diseases including viral bacterial and fungal. These medicinal plants have future aspects because of the activity against *Aspergillus* and *E. coli*.

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