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From Garden to Lab: Harnessing Antimicrobial Strengths in Selected Plants

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Abstract

Worldwide, plants are utilized as natural sources of bioactive chemicals, which are employed as antibacterial agents. Due to the increase in the antimicrobial resistance against current antibiotics, there is a need of develop new antimicrobial compounds or extracts. Numerous secondary metabolites are found in plants that have been shown to exhibit antibacterial activities. In the present studies, several locally growing plants were tested for their ability to inhibit both gram-positive and gram-negative bacteria. Study of selected plants showed good activity against tested bacteria, with a minimum inhibitory concentration of 10mg/ml for *E. coli* and *S. aureus*. Very few plants showed growth inhibition in *L. rhamnosus* at 15mg/ml while others were non-toxic upto a dose of 100mg/ml. The study demonstrated the antimicrobial potential of the screened plants. The results signified that tested plants could be a potential source of future drugs for combating bacterial infections, without harming good bacteria of gut microbiome.

Introduction

The development of new drugs plays a crucial role in our ability to treat diseases, and medicinal plant offers a potential avenue for discovering novel drugs. The anti-microbial properties in plants are capable of inhibiting bacterial, fungal, and viral growth by distinct mechanisms than presently used antibiotics and are considerably beneficial for treating resistant bacterial strains [1]. Due to an increase in antibiotic resistance, compounds obtained from plants are explored and checked for their antibacterial activity. Numerous secondary metabolites found in plants, including flavonoids, terpenoids, alkaloids, and tannins, have been shown to exhibit antibacterial qualities in vitro [2]. Being the natural origin plants have a positive influence on the environment and are utilized as biological control agents. Plants have shown a prominent role in treating illnesses and infections in humans; in both traditional and modern medical systems. Due to which the demand for plant-based medicine has increased as a result of the greater awareness of herbal products. Out of all the anti-microbial compounds available in today's world, plant products occupy the major part [3]. As per WHO reports, more than 80 percent of people in the developing world utilizing plants as a source of traditional medicine [4]. There are thousands of antimicrobial compounds isolated from plants, and more than 1340 plants have been known to demonstrate definite anti-microbial activity [5]. The bioactive compounds produced by plants play a vital part in abiotic stress management for plants, as well as in humans due to their use in pharmaceutical industries [6,7]. The currently available antibiotics have become less effective because of the rise in antibiotic resistance [8]. The World Health Organisation defines antibiotic resistance as the result of microorganisms, such as bacteria, fungi, viruses, and parasites, changing over time to become resistant to antibiotics. This makes illnesses harder to treat and raises the risk of serious sickness and the spread of disease [9]. Due to the emergence of antibiotic resistance, some antibiot-

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ics have become ineffective and are not capable of inhibiting bacterial growth leading to spread of infections and illness. Currently antimicrobial resistance would kill 10 million people worldwide each year, and would cost the global economy US$ 100 trillion [10]. Gram negative bacterium like *Escherichia coli* is facultative anaerobic, belonging to the family Enterobacteriaceae that typically establishes itself in both warm-blooded animals' and humans' digestive systems only after few hours of birth [11]. Normally, commensal *E. coli* strains do not lead to illness unless the host's immune system is compromised or the gastrointestinal barriers are breached. Some strains are capable of causing illnesses that are sometimes severe, such as diarrhea, respiratory disorders, urinary tract infections, and bloodstream infections [12]. According to one hypothesis, *E. coli* could take advantage of its superior capacity to metabolize gluconate in the colon, outperforming other bacterial species present in the same environment [13]. Due to its unique characteristics, its ease of handling, availability of the full genome sequence, and ability to grow in both aerobic and anaerobic environment, *E. coli* is a valuable bacterium for use in research labs and the field of recombinant DNA. Treating *E. coli* infections has become increasingly challenging due to the rise in resistance of the majority of first-line antibiotic medications [14]. Due to the widespread use of antibiotics, bacterial populations are under selective pressure, which leads to the emergence of drug resistance or multidrug resistance [15]. The main cause of growing cephalosporin resistance, according to reports of antibiotic resistance in the Enterobacteriaceae family worldwide, is the expansion of Extended Spectrum β Lactamse (ESBL) [16]. Resistant *E. coli* strains have the ability to spread antibiotic resistance genes to other gastrointestinal tract bacteria as well as to other strains of the same bacterium [17].

In response to the situation, researchers have been actively exploring alternative sources to discover new antimicrobials for treating *E. coli* infections, they have been investigating various sources, including medicinal plants, in search for novel antimicrobial therapeutic agents. The reason behind that is synthetic drugs, which are costly to produce and often have undesirable harmful effects, compared to plant derived medicines. Bioactive compounds from plants exhibit both antibiotic resistance-modifying and innate antibacterial action. Even though some compounds are not as effective as antibiotics on their own, but their combined activity with antibiotics can greatly enhance their activity in overcoming bacterial resistance towards antibiotics. Due to this the synergistic potential of medicinal plant is tested with antibiotics [18]. The most widely used techniques for evaluating the antimicrobial activity of plant extracts are broth and agar dilution methods tests, agar well diffusion, and disk-diffusion assay [19]. This extensive investigation uses agar well diffusion to assess the inhibitory potential of twenty-three selected plants.

Material and methods

Reagents & Chemicals

LB Media, LB agar, MHA, MRS, PBS, Antibiotics, were purchased from Himedia, All the other chemicals used were of analytical grade.

Plant material collection and preparation of samples

Plant materials were collected from their natural habitat or procured, and whole plants materials were wild type requiring no licences for the applications. The freshly harvested material was properly cleaned and dried. Dried samples were ground using a mortar pestle to result in a fine powder. Each sample was incubated in 90% ethanol for 72 hours in a 1:10 ratio. Following that, each was centrifuged for 10 minutes at 9,500 rpm. The resultant solution was filtered with Whatman's filter paper and the filtrate was evaporated using a Rotatory evaporator at 40°C temperature and under low pressure and stored at 4°C until analysis [20].

Antibacterial assay

The antibacterial studies of plants were conducted on *E. coli* (DH5α), *L. rhamnosus,* and *S. aureus.* The antibacterial activity of different plant species was evaluated by agar well diffusion method utilizing Mueller-Hinton Agar and deMan Rogosa Sharpe Agar. The respective microbial cultures were activated by inoculating a loopful of the strain in the Luria Bertini broth medium for *E. coli* and *S. aureus* and MRS broth for *L. rhamnosus* and incubated at 37°C in shaker incubator using standard methodology [21]. The test samples were incubated into the freshly made well respectively and the plates were incubated at 37°C for 24h. Each experiment was performed 3 times, under aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of the inhibition and the mean values were recorded. Antibiotics streptomycin (100µg/ml) was used as positive control for *E. coli*, tetracyclin (100µg/ml) was used for *L. rhamnosus* and ampicillin (100 µg/ml) for *S. aureus*. Water was used as a negative control. All the data were expressed as Mean ± SD, of three independent replications.

FTIR Spectroscopy: FTIR spectra of plant samples were measured with ATR, Bruker ALPHA, Germany in the range of 500-4000 cm^{-1}.

Results

The FTIR spectra of plants were recorded to ascertain the functional groups of the selected plants responsible for antibacterial activities (Figure 1-5). FTIR spectroscopy data from species of cinnamon showed band similarities in CH, CH2 and CH3 stretching, C=C, C-O functional groups, and dissimilarity in C-N, O-H functional groups which was showed by only cinnamon (P) and *Cinnamomum zeylanicum* (Ceylon cinnamon), whereas COO, C-C, COO, NH, CO^{2}_{3-} functional groups reported in case of *Cinamon cassia*, COOH function group found in *Cinnamon zeylanicum* and *Cinnamon verum*, NH group showed by all the three species of cinnamon excepting cinnamon (P). FTIR spectra of *Neolamarckia cadamba* (Figure 2), *Curcuma longa* (Figure 3), *Withania somnifera* (Figure 4), and *Terminalia arjuna* (Figure 5) showed similarity in CH, CH2, CH3 stretching, C-O, C-N, O-H functional groups. C=C functional group found only in *C. longa* and *N. cadamba*. C-N group recorded in all the three plants except in case of *W. somnifera*. On the other hands, N-H and C=O functional groups reported in the case of *C. longa*.

The antibacterial activity of plant extracts on *E. coli*, *L. rhamnosus* and *S. aureus* strains showed significant inhibition of bacterial growth. For *L. rhamnosus* strain 23 plants out of 12 plants showed the growth inhibition. *Syzygium cumini* (22 mm), and *Terminalia arjuna* (22 mm) showed the largest zone of inhibition followed by *Cinnamon zeylanicum* (18 mm). Plants *Manilkara zapota*, *Brassica oleracea*, *Withania somnifera*, *Tecoma stans*, *Ocimum sanctum, Tinospora cordifolia*, *Ocimum tenuiflorum*, *Tectona grandis*, *Fxaxinus excelsior*, *Azadirachta indica, and Saraca asoca* did not show anti-inhibitory activity upto concentration 100 mg/ml (Figure 6,11). In case of *E. coli,* a total

of 22 plants out of 23 inhibited growth and the largest zone of inhibitions showed with *Fraxinus excelsior* (28 mm) followed by *Cinnamomum zeylanicum* (24 mm), *Cinnamomum verum* (23 mm), *Cinnamomum cassia* (22 mm), cinnamon (P) (20 mm), *Neolamarckia cadamba* (20 mm) and *Azadirachta indica* (20 mm), while *Tinospora cordifolia* didn't show inhibitory activity (Figure 7,8, 9). For *S. aureus*, five plants were studied which showed good inhibitory zone with respect to *E. coli* and *L. rhamnosus*, *Cinnamomum cassia*, shows the larger zone of inhibition of 22 mm followed by *Cinnamomum zeylanicum* (20 mm), *Cinnamomum verum* (20 mm), powered cinnamon (19 mm) and *Neolamarckia cadamba* (16 mm). The study found that the growth of *E. coli* colonies were inhibited effectively with Cinnamon sps, *Neolamarckia cadamba*, *Syzygium cumini*, *Manilkara zapota*, *Glycyrrhiza glabra*, *Fxaxinus excelsior*, *Punica granatum* and *Clitoria ternatea*. Whereas *Glucyrrhiza glabra*, *Terminalia arjuna*, *Punica granatum,* and *Syzygium cumini* were effective as antimicrobial agent against *L. rhamnosus*. A separate study was done with different species of Cinnamon and *Neolamarckia cadamba* with *S. aureus*, *E. coli,* and *L. rhamnosus*, it was found that tested plants were cytotoxic for *E. coli* and *S. aureus* at 40, 20 and 10mg/ml while these concentrations were not effective on the colonies of *L. rhamnosus* (Figure 9,10,11).

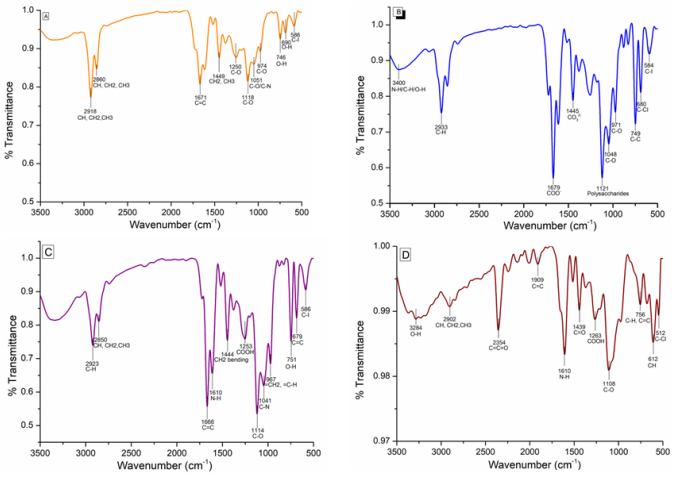

Figure 1: Fourier Transform Infrared Spectroscopy graph of **(A)** Cinnamon species (P) **(B)** *Cinnamomum cassia* **(C)** *Cinnamomum zeylanicum* **(D)** *Cinnamomum verum.*

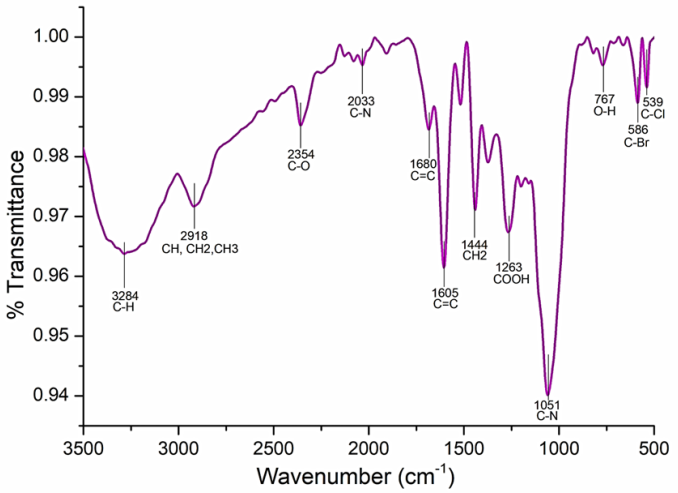

Figure 2: Fourier Transform Infrared Spectroscopy graph of *Neolamarckia cadamba.*

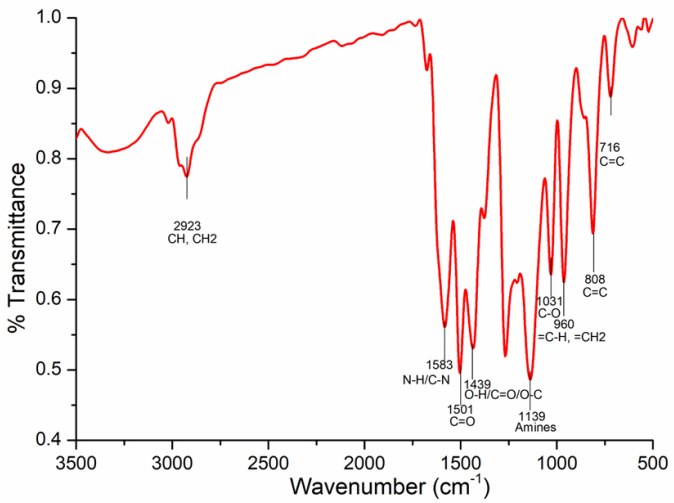

Figure 3: Fourier Transform Infrared Spectroscopy graph of *Curcuma longa.*

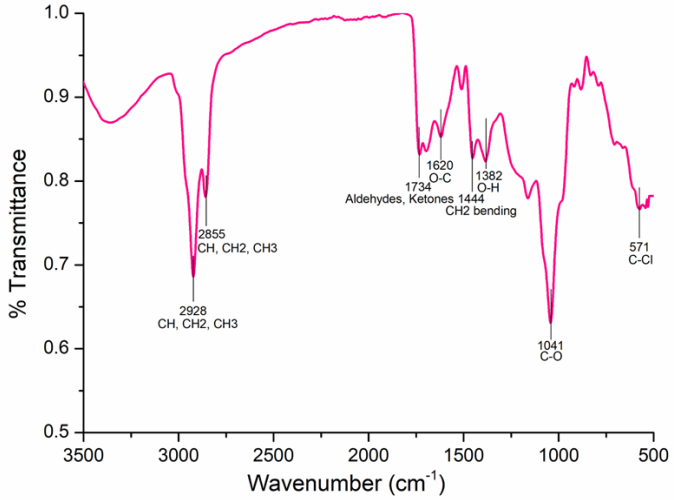

Figure 4: Fourier Transform Infrared Spectroscopy graph of *Withania somnifera.*

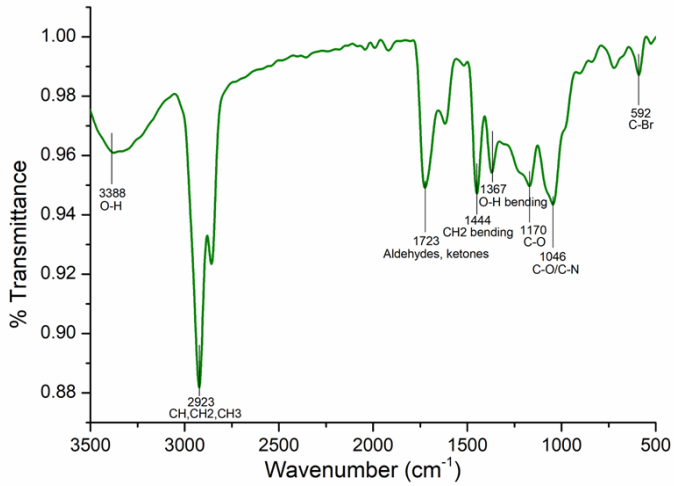

Figure 5: Fourier Transform Infrared Spectroscopy graph of *Terminalia arjuna.*

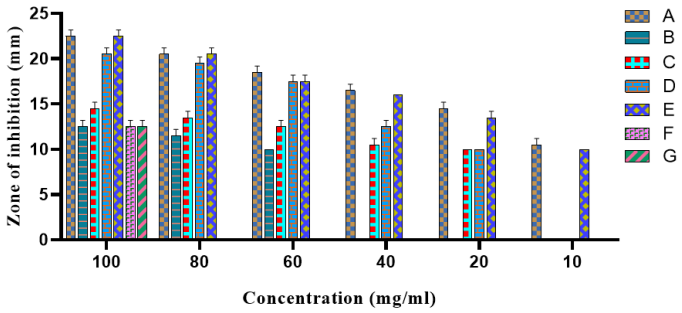

Figure 6: Studies of the plants in dose-dependent manner with *Lactobacillus rhamnosus*. **(A)** *Saraca asoca;* **(B)** *Cucurma longa;* **(C)** *Glycyrrhiza glabra;* **(D)** *Terminalia arjuna;* **(E)** *Punica granatum*; **(F)** *Asparagus racemosus;* **(G)** *Clitora ternatea*. Data expressed as Mean ± SD.

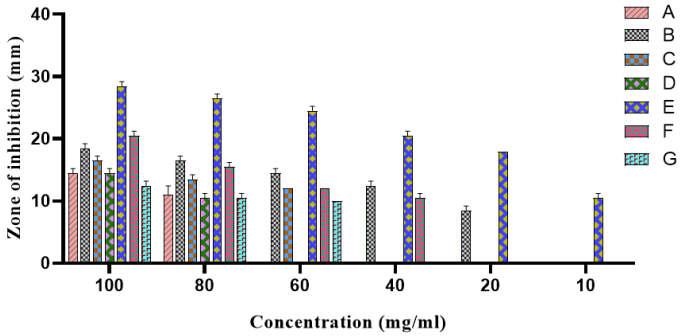

Figure 7: Studies of the plants in dose-dependent manner with *Escherichia coli* (DH5α). **(A)** *Ocimum sanctum* **(B)** *Punica granatum* **(C)** *Ocimum tenuiflorum* **(D)** *Tectona grandis* **(E)** *Fraxinus excelsior* **(F)** *Azadirachta indica* **(G)** *Saraca asoca*. Data expressed as Mean ± SD.

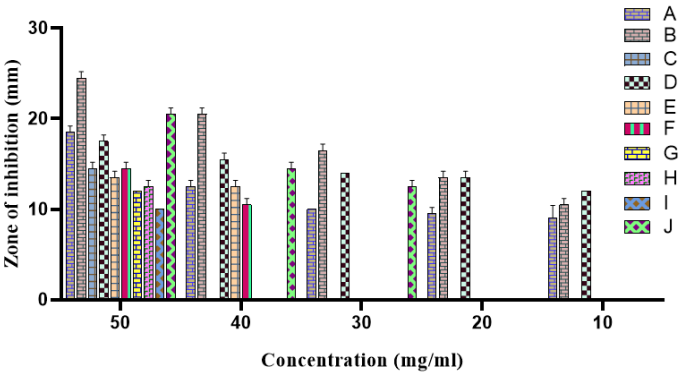

Figure 8: Studies of the plants in dose-dependent manner with *Escherichia coli* (DH5α). **(A)** *Syzygium cumini*; **(B)** *Manilkara zapota*) **(C)** *Curcuma longa*; **(D)** *Glycyrrhiza glabra* **(E)** *Brassica oleracea* **(F)** *Withania somnifera* **(G)** *Tectona stans* **(H)** *Terminalia arjuna* **(I)** *Asparagus racemosus* **(J)** *Clitoria ternatea*. Data expressed as Mean ± SD.

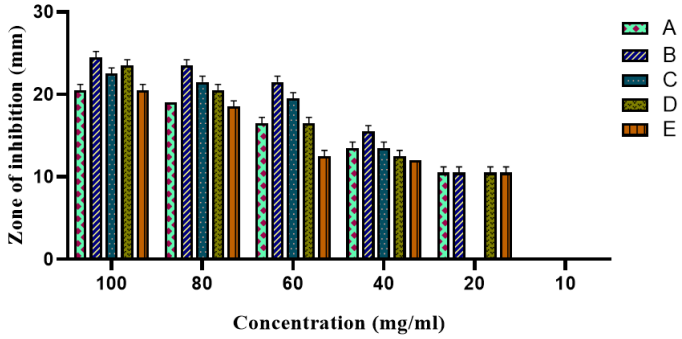

Figure 9: Studies of the plants in dose-dependent manner with *Escherichia coli* (DH5α). **(A)** Cinnamon species (P); **(B)** Ceylon cinnamon (*Cinnamomum zeylanicum*); **(C)** *Cinnamomum cassia*; **(D)** *Cinnamomum verum*; **(E)** *Neolamarckia cadamba.* Data expressed as Mean ± SD.

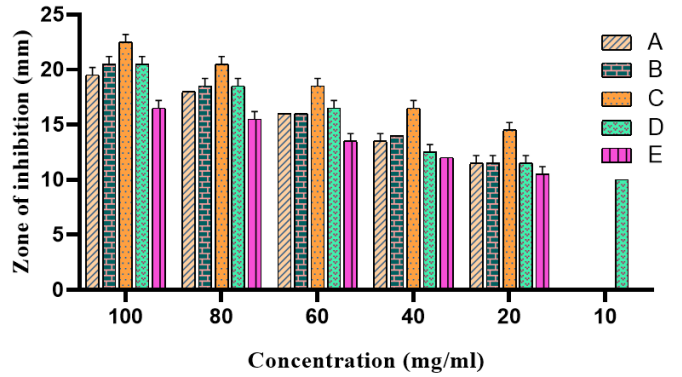

Figure 10: Studies of the plants in dose-dependent manner with *Staphylococcus aureus*. **(A)** Cinnamon species (P); **(B)** Ceylon cinnamon (*Cinnamomum zeylanicum*); **(C)** *Cinnamomum cassia*; **(D)** *Cinnamomum verum*; **(E)** *Neolamarckia cadamba.* Data expressed as Mean ± SD.

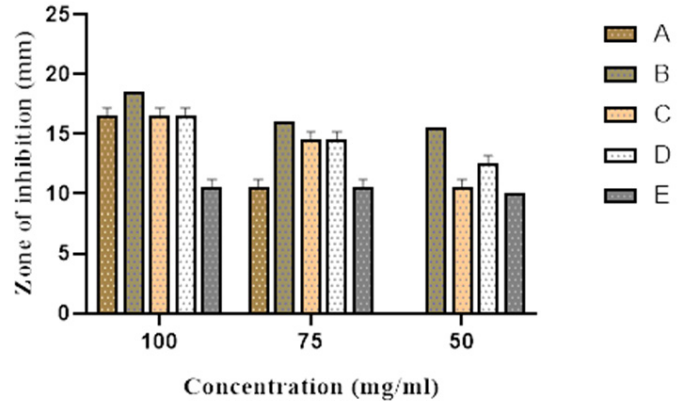

Figure 11: Studies of the plants in dose-dependent manner with *Lactobacillus rhamnosus*. **(A)** Cinnamon species (P); **(B)** Ceylon cinnamon (*Cinnamomum zeylanicum*); **(C)** *Cinnamomum cassia;* **(D)** *Cinnamomum verum*; E) *Neolamarckia cadamba.* Data expressed as Mean ± SD.

Discussion

The goal of the current investigation is to ascertain the potential of the antibacterial activity in plants. The study is important in the light of the microbial population's increasing resistance to commercially available antibiotics. Researchers are trying to assess various medicinal plants as an antibiotic substitute due to rise of resistant strains against widely used antibiotics leading to MDR (Multiple drug resistance). Many plant species have antibacterial properties due to the presence of potent phytochemicals [22-24]. In the present study, the antimicrobial potential of twenty-three plants belonging to different families were targeted against gram-positive and gram-negative bacteria, using well diffusion method at varying doses ranging from 1.0 mg to 100 mg of plant. The results of the selected plants were presented in Figures 6-11. Bioactive compounds like flavonoids, phenols, anthocyanin, ascorbic acid, amides, alkaloids, tannins, saponins, and glycosides, from different parts of medicinal plant species play an important role in improving human health, due to their biological activity such as anti-oxidant, anti-cancer and other

pharmacological activities [23-25]. The antibacterial activity is highly variable for the plants showing the anti-bacterial activity arises due to different concentrations and interactions of these metabolites with other components. Our research findings indicated that several selected plants significantly inhibited the growth of bacteria; the plants that were most significant in this regard were *Punica granatum*, *N. cadamba*, *S. cumini, M. zapota,* and cinnamon species. The majority of bioactive molecules from plants that have therapeutic effects are categorised as secondary metabolites [23,24]. Plant secondary metabolism produces secondary metabolites which is highly networked and complex pathway with primary metabolism [26]. The structure, quantity, and location of substituent groups, the existence of glycosidic connections, the alkylation of OH groups, the geography and climate of the area of origin, chemotypic variations and other factors all contribute to their broad range of phytotherapeutics and antimicrobial action [27-29]. The antibacterial action of bioactive secondary metabolites varies depending on the kind and concentration of the microbial strains they affect [30]. Bioactive substances found in plants such as flavonoids, phenols, alkaloids, tannins, saponins, and glycosides, have been shown to be crucial for various pharmacological properties including antibacterial activity [28]. Alkaloids are heterocyclic nitrogen molecules with extremely varied chemical structures that have antibacterial, analgesic, and spasmodic properties. Among the most varied classes of bioactive secondary metabolites found in medicinal plants are phenolic phytochemicals followed by terpenoids specially monoterpenoids and sesquiterpenoids [28,31]. They are often used to combat infectious diseases. The known mechanisms include the inhibitory activity of the efflux pump, the ability to alter the permeability of cell membranes, the shifting of several intracellular functions due to the binding of phenolic compounds to enzymes, also the loss of integrity of the cell wall as a result of various interactions with the cell membrane. Specifically, flavones are an antibacterial agent that can break down microbial envelopes and disrupt particular microbial enzymes [32]. A wide range on study showed that the essential oils derived from the plants also have great antimicrobial properties [28,33]. The Minimum Inhibitory Concentration (MIC) of the plants were also discovered for the bacterial strains under investigation. The study's conclusions are given, and they include helpful information on possible application for the plants as antibacterial qualities [34]. Turmeric have been demonstrated to be effective against *S. aureus* and *E. coli* because they contain the phenolic molecules curcuminoid. *Curcuma longa* had good antibacterial action against a number of harmful microbes, including *S. aureus* and *E. coli* [35,36]. *S. cumini* reported to have antibacterial activity against both gram positive and gram-negative bacteria [21]. *Cinnamomum cassia* essential oils and its components, *Cinnamomum zeylanicum* and *Cinnamomum loureiroi* showed antibacterial activity in earlier studies against *S. aureus* and *E. coli* [33,37-39]. *N. cadamba* exhibited antimicrobial action against *E. coli* and *S. aureus* [40]. Methanolic fruit extract of *N. cadamba* has been shown to inhibit antibacterial against *Klebsiella pneumonia* [41], similarly *G. glabra* root showed less inhibitory activity against *S. aureus* and *E. coli* at much higher concentrations towards these strains [42]. According to previous study showed antimicrobial activity in dose dose-dependent manner [43]. *P. granatum* peel showed MIC at 200 µg/ml in case of *E. coli* [44]. *A. racemosus* significantly inhibited the growth of gram-positive bacteria *E. coli* [45]. *C. ternatea* showed an average ZOI with *E. coli* at 200 µg/ml [46]. Cinnamon and *N. cadamba* showed a good antimicrobial activity against *E. coli* and *S. aureus* that can be used as antimicrobial agents up to 40 mg/ml as this concentration was found to be nontoxic for good bacteria such as *L. rhamnosus.* The current study's findings showed that the type of plant and plant material utilised affect the antibacterial activity. It is also implied that every plant has its own unique profile of chemicals, though some commonalities may exist. The uniqueness in phytochemicals and their profiling may help in ascertaining specific group based plants. The groups are in turn linked, in general to their ability for anti-microbial properties. Also, the study determined the usefulness of the plants, which may be highly relevant to the creation of safe medications as antimicrobials from plants.

Conclusion

On the basis of antibacterial assay in this study, selected plants showed good inhibitory activity. *S. cumini, F. excelsior, M. zapora, G. glabra* and *C. verum* plant showed highest inhibitory potential at 100 mg/ml, while *T. cordifoia* was not effective antibacterial agent upto in case of *E. coli*. *S. cumini* and *P. granatum* inhibited the growth of *L. rhamnosus* at 15 mg/ml, many of the tested plants didn't affect growth of bacteria which may be required as part of gut microbiome. Cinnamon and *N. cadamba* also tested on *S. aureus* and showed significant growth inhibition at 10-20 mg/ml while the dose was not toxic for *L. rhamnosus*. The investigation proved these plants as a potential antibacterial agent. The findings suggest that while *L. rhamnosus* was not as much inhibited by plants, *E. coli* and *S. aureus* were effectively inhibited. The plants can be further analysed for characterizing the specific anti-bacterial component and other parts of plant like root, stem, flower, and fruit can also be tested for their inhibitory potentials and toxicity studies. Studies should also be conducted to check the safety level of selected plants. The search results also include other studies that evaluated the antimicrobial activity of plants against various bacterial strains, highlighted the potential of natural products in compacting microbial infections.

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