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ANTIMICROBIAL POTENTIAL OF ETHANOLIC EXTRACTS OF AVACADO, ALLSPICE, TEJPATTA AND DALCHINI AGAINST DIFFERENT BACTERIAL STRAINS

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ABSTRACT

Microbial activity causes serious damage to living organisms. In this study, ethanolic extracts were prepared from leaves of *Persea americana* Mill. *Pimenta dioica* (L.) Merr., *Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm. and *Cinnamomum verum* J. Presl. The ethanolic extracts prepared from these plants exhibited broad spectrum antibacterial activity when evaluated against gram-positive bacteria *Streptococci* spp. and *Staphylococci aureus* and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion method and growth inhibition assay.

KEYWORDS: *Staphylococcus aureus*, *Persea americana*, *Pimenta dioica*, *Pseudomonas aeruginosa* and antimicrobial.

INTRODUCTION

Plants have become the center of interest for the cure of various diseases in recent past. The utilization of plants for treatment of any disease has been tremendously increased from the last few years, because of the wider belief of less adverse effects. The risk of infection is increasing progressively, due to growing environmental pollution and lifestyle. This also leads to mutation and drug resistance bacteria. Whereas, synthetic antimicrobial agents that are used are also having many side effects and cause harm to the human body. Therefore, several natural products based antimicrobials have emerged to tackle this problem.

Spices are flavored or aromatic substances originating from vegetables and are obtained from the topical plants and are used to enhance the taste of the food and as condiments. The spices contain enormous amount of volatile oil which gives a general notion of the antimicrobial potential of various spices. This made the spices to utilize as adjuvant as antimicrobial agents for controlling the risk of infection. In the present study, four different plant leaves were collected and investigated for anti-microbial activity. The plant details such as botanical names, family, common name and parts used in the study are given in table 1.

Table 1: Plants Name and its description.

Botanical Name	Family	Common name	Part used
Persea americana Mill., & Eberm	Lauraceae	Avocado	Leaf
Pimenta dioica (L.) Merr.	Myrtaceae	Allspice	Leaf
Cinnamomum tamala (Buch.-Ham.) T.Nees	Lauraceae	Bay leaf, tejpatta	Leaf
Cinnamomum verum J. Presl	Lauraceae	Cinnamon, dalchini	Leaf

***Persea americana* (Avocado)**

Avocado belonging to the family of Lauraceae and its botanical name is *Persea americana* is a tropical tree crop originating from tropics of the western hemisphere. The avocado originates in highlands of the Guatemalan, and central and east-central Mexico highlands. The height of the Avocado tree reaches 30 m (Guatemalan and West Indian species) and the diameter of the trunk between 30 - 60 cm. The leaves of the plant are dark-greenish in color with a glossy layer on the upper surface, lanceolate, elliptic, ovate, and are alternatively arranged on the stem. The plant contains fruits of pear-

shaped and the greenish yellow pulp of fruit is edible.[1] The largest producer of avocado is Mexico with about 25% of the world production.[2] The traditional system of medicine reported good medicinal properties of Avocado which include antiulcer effect, anticonvulsant activity, antihepatotoxic activity, hypoglycemic activity and antioxidant activity.[3]

***Pimenta dioica* (Allspice)**

Allspice scientifically known as *Pimenta dioica* is a member of family Myrtaceae. It is an evergreen, tall and bushy tree of height 6.6 - 10 m. It is more branched at the top and the color of the bark is greyish. Leaves are oppositely arranged, oblong, lanceolate, tapering toward petioles and the size of leaves varies to 11-11.5 cm X 5 - 6 cm and it contains essential oils.[4] Allspice is indigenous to West Indies and Central America. The essential oils of the plant are like that of clove, nutmeg, cinnamon, and black pepper, so the effect produce by allspice is somewhat similar to these. It contains bilocular berries which are hard, brown-reddish having a diameter of 4 – 7 mm. Berries have a characteristic odor because of the essential oils present in it. Allspice contains phytoconstituents like alkaloids, glycosides, flavonoids, amino acid, saponins, tannins, etc. The plant acts as a very strong bacteriocide because of the presence of spice components which are thymol, eugenol, menthol and anethole. It also acts as an insecticide and antioxidant and is used as a food preservative. Other effects of allspice are the same as that of clove and other spice. It is also used as a digestant and stomachic.[5]

***Cinnamomum tamala* (Tejpatta)**

Tejpatta botanically known as *Cinnamomum tamala* of family Lauraceae is a small or perennial evergreen tree of height 8 – 12 m, the stem is rough with a girth of 150 cm and produces mucilage. Leaves are long (about 12 – 20 cm) and are 5 - 8 cm broad. Its shape is somewhat ovate-lanceolate, thick, leathery, shining green on top and is oppositely arranged. The flower of the plant blossoms at the end of March or at the starting of April. Its leaves are vastly used as a spice for enhancing the flavor of food. The oil of tejpatta obtained from leaves is used as a diuretic, carminative and in cardiac disorders. It has been used as a food preservative for pineapple juice. Its special aroma makes it useful in the food industry. The main constituents of *C. tamala* are camphene, pinene, limonene and eugenol. Traditionally, tejpatta leaves are used in bladder disease, diarrhea, mouth dryness and nausea. It also shows hypoglycemic and hypolipidemic activity.[6]

***Cinnamomum verum* (Dalchini)**

The botanical name of dalchini is *Cinnamomum verum* and to the family of Lauraceae. It is widely found in South India and Sri Lanka. It is the oldest tree spice that was used by mankind. It is an evergreen, medium-sized tree of height about 16 m. Its bark has a delicately fragrant aroma with warm and sweet flavor along with pungent taste. Cinnamon leaves are shiny green on the upper side and have a leathery look. The lower side is somewhat dull than the upper. When rubbed in hands, its leaves emit some spicy odor. Leaves are oppositely arranged, oval or elliptic to lanceolate and length of the leaves varies from 8.7 - 22.7 cm. It contains bisexual flowers which are pale yellowish-green in color and flowering occurs in October or November and continues till March [7]. Cinnamon or dalchini is the most commonly used spices that enhance food's flavor and is also used in tea as a taste enhancer. The small quills of its bark have a huge market all over the world because of the presence of chemical constituent oleoresin. The dried dalchini leaves are also used for food flavoring. It shows hypoglycemic activity and can be used for treating type 2 diabetes mellitus. The presence of phenolics *t*-cinnamaldehyde, eugenol and cinnamic acid are responsible due to its antioxidant and antimicrobial property, for which it is used as a food preservative. The plant is reported for its antifungal, antibacterial and insecticidal activity.[8]

MATERIALS AND METHODS

In the current study plant extracts were prepared and evaluated for anti-microbial activity by the disc diffusion method and growth inhibition assay. The plants selected for the study were Persea americana (Avocado), Pimenta dioica (Allspice), Cinnamomum tamala (Tejpatta) and Cinnamomum verum (Dalchini) (Details are given in table 1).

Plant material collection

Fresh leaves of all four plants were collected from the Madan Mohan herbal garden of Jawaharlal Nehru Cancer Hospital and Research Centre, Idgah Hills, Bhopal, India.

Preparation of plant extract

The leaves collected from four plants were cleaned from both sides properly with tissue paper and kept aside for a few minutes to dry at room temperature. The leaves were minced in small pieces using sterilized scissors. The mined leaves were soaked in 40 ml of 50% ethanol (50 ml ethanol and 50 ml distilled water) in a clean beaker for 24 hours. The extracts were centrifuged after 24 hours for 15 minutes at 1000 rpm. The prepared extract was used for further experiments and the rest of the extract stored at 4°C in the refrigerator.

Antimicrobial Activity

The antimicrobial activity was tested in plant extracts by growth inhibition assay and disc diffusion method using different bacterial strains.

Bacterial strains

The bacterial strains tested for the antibacterial activity were isolated in the Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre. The strains used for the study were two gram-positive bacteria *Streptococci* spp. and *Staphylococci*

aureus, whereas *Pseudomonas aeruginosa* and *Escherichia coli* were used as gram-negative bacteria.

Disc Diffusion Method

The plant extracts were examined for antimicrobial activity by using disc diffusion method. The discs were taken in different petri plates and loaded with extracts and air-dried and subsequent loading was done with drying. After this, the discs were taken in glass vials, labeled and autoclaved. The discs for negative control were also prepared in ethanol only. The bacterial strains were inoculated on Mueller Hinton plates using sterile stirrer of high-quality stainless steel. After inoculation, the extract discs were placed using sterile forceps. The negative and positive control discs were also put on plates and were incubated at 37°C. After 24 hours of treatment, the plates were observed and the zone of inhibition was calculated. For positive control, standard discs were placed separately for gram negative and gram positive bacteria.[9]

Growth Inhibition Assay

Nutrient agar media was prepared and poured in plates with 500 µl of ethanolic extract and mixed using stirrer. The media was allowed to solidify and after solidification bacterial strains were inoculated using swab and incubated at 37°C. The plates were examined after 24 hours of treatment and the number of bacterial colonies was counted.[10]

RESULTS

In the present study four plants were selected which includes *P. americana*, *P. dioica*, *C. tamala* and *C. verum*. Disc diffusion method and growth inhibition assay was performed to examine antimicrobial potential of ethanolic extract of all four plants. The phytochemical like flavonoids and tannins present in plants, were responsible for its antimicrobial activity.

Disc Diffusion Method

The ethanolic extracts of plants was loaded on discs and placed on Mueller Hinton plates showed potent antimicrobial activity against all strains used for the study. The strains used for the study were *P. aeruginosa*, *Streptococci* spp., *S. aureus* and *E. coli.* *P. americana* (Avacado) showed zone of inhibition of 7 mm, 8 mm and 12 mm against *Streptococci* spp., *P. aeruginosa* and *S. aureus* respectively. *P. dioica* (Allspice) were found more effective than other plant extracts and gave zone of inhibition of 6 mm, 8 mm, 8 mm and 18 mm against *E. coli*, *P. aeruginosa*, *Streptococci* spp., and *S. aureus* respectively. The extract (ethanolic) of *C. tamala* and *C. verum* was found inactive and no zone of inhibition was found against both the plant extracts. The zone of inhibition obtained in standard disc used for the study for gram positive (Table - 3) and gram negative (Table - 4) bacteria were given in tabular form for comparative study. The results of extracts by disc diffusion method found were also given in Table -2.

Growth Inhibition Assay

The nutrient agar plates were taken along with 500µl of extract and inoculated with bacterial strains. The extracts which inhibited the growth of bacteria showed less or no growth in culture plates (Table -5). The ethanolic extracts of *P. americana*, *P. dioica*, *C. tamala* and *C. verum* leaves show potent antibacterial activity against *P. aeruginosa*, whereas *P. americana* inhibited the growth of *Staphylococcus aureus* bacteria.

Table 2: The zone of inhibition of ethanolic plant extracts against *Streptococci, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. The diameter of zone of inhibition (mm). No zone of inhibition indicated by NZ.

S. No.	Plant Name	Zone of Inhibition (mm)			
		Streptococci	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***
1.	Avocado	7 mm	8 mm	12 mm	NZ
2.	Allspice	8 mm	8 mm	18 mm	6 mm
3.	Tejpat	NZ	NZ	NZ	NZ
4.	Dalchini	NZ	NZ	NZ	NZ

Table 3: Standard Drug for Gram positive bacteria.

No.	Drug Name	Zone of Inhibition (mm)	
		***Streptococci* spp.**	***Staphylococci aureus***
1.	Cefotaxine	NZ	-
2.	Ceftriaxome	NZ	-
3.	Ceftazidine	NZ	-
4.	Nitrofurantoin	NZ	-
5.	Gentamicin	NZ	-
6.	Amikacin	NZ	-
7.	Cefuroxime	20 mm	-
8.	Cefixime	NZ	-
9.	Nalidixic acid	NZ	-

10.	Cefdinir	NZ	-
11.	Cephalexin	18 mm	-
12.	Tetracyclin	10 mm	-
13.	Piperacillin	30 mm	-
14.	Pinicillin G	16 mm	-
15.	Cefazolin	16 mm	-
16.	Chloramphenicol	14 mm	-
17.	Ciprofloxacin	7 mm	-
18.	Erythromycin	13 mm	-
19.	Azithromycin	12 mm	-
20.	Cotrimoxazol	18 mm	-
21.	Amoxicillin clavulanic acid	25 mm	-
22.	Amoxicillin	25 mm	-

Table 4: Standard Drug for Gram negative bacteria.

S. No.	Drug Name	Zone of Inhibition (mm)	
		Escherichia coli	***Pseudomonas aeruginosa***
1.	Cefotaxine	-	23 mm
2.	Ceftriaxome	-	20 mm
3.	Ceftazidine	-	12 mm
4.	Nitrofurantoin	-	18 mm
5.	Gentamicin	-	12 mm
6.	Amikacin	-	10 mm
7.	Cefuroxime	-	18 mm
8.	Cefixime	-	8 mm
9.	Nalidixic acid	-	8 mm
10.	Cefdinir	-	20 mm
11.	Cephalexin	-	-
12.	Tetracyclin	-	-
13.	Piperacillin	-	-
14.	Pinicillin G	-	-
15.	Cefazolin	-	-
16.	Chloramphenicol	-	-
17.	Ciprofloxacin	-	-
18.	Erythromycin	-	-
19.	Azithromycin	-	-
20.	Cotrimoxazol	-	-
21.	Amoxicillin clavulanic acid	-	-
22.	Amoxicillin	-	-

Table 5: Growth inhibition Assay of ethanolic plant extracts against *Streptococci* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

S. No.	Plant Name	Bacterial Strains				
		Streptococci	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***	***Streptococci***
1	Avocado	Moderate growth of bacteria	No growth	No growth	Inhibition not present	Inhibition not present
2	Allspice	Inhibition not present	No growth	Inhibition not present	Inhibition not present	Inhibition not present
3	Tejpat	Moderate growth of bacteria	No growth	Inhibition not present	Inhibition not present	Inhibition not present
4	Dalchini	Inhibition not present	No growth	Moderate growth of bacteria	Inhibition not present	Inhibition not present

Figure 1: Plants common and botanical name. (A) *Persea americana* (Avocado), (B) *Pimenta dioica* (Allspice), (C) *Cinnamomum tamala* (Tejpatta) and (D) *Cinnamomum verum* (Dalchini).

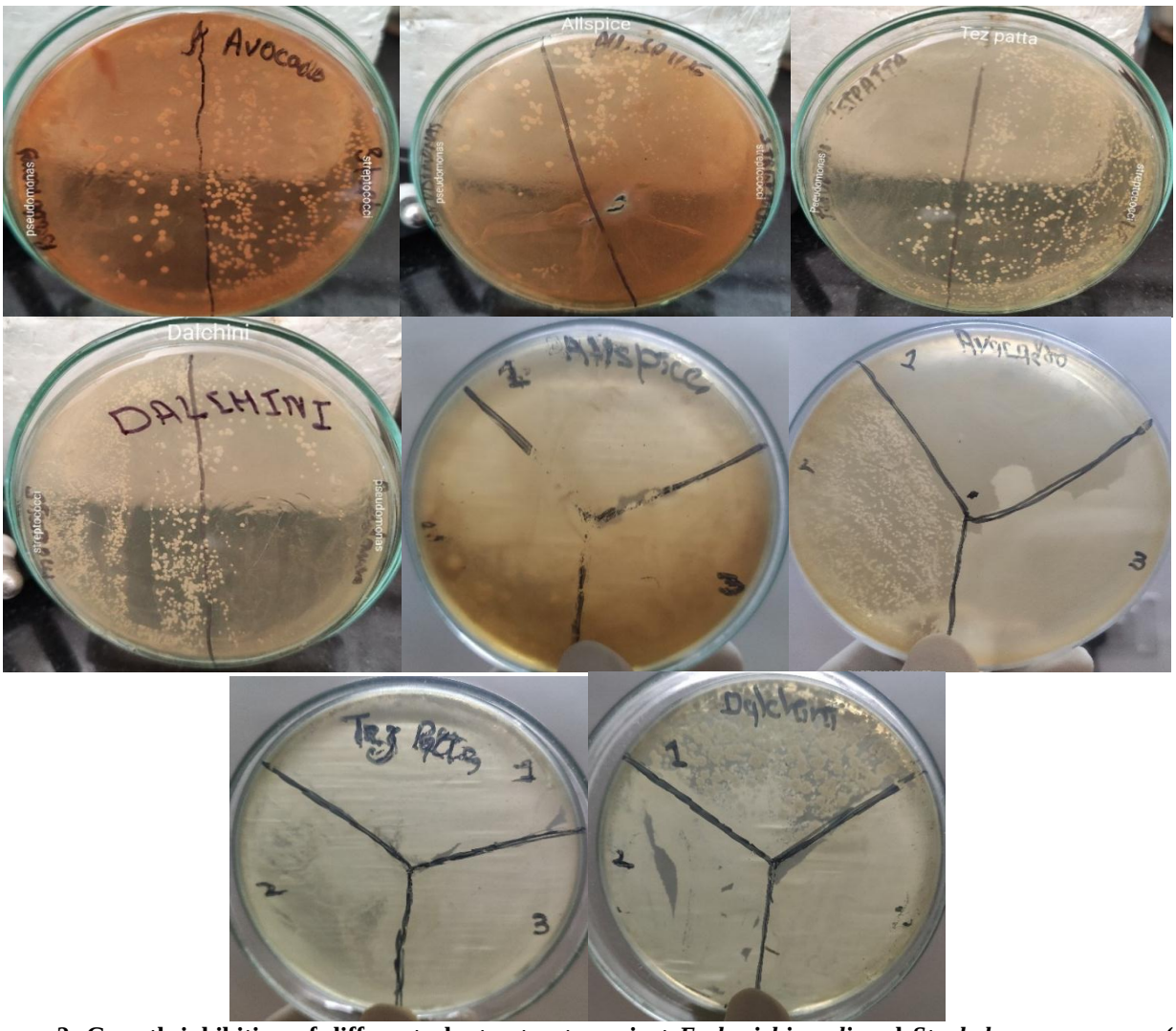

Figure 2: Growth inhibition of different plant extracts against *Escherichia coli* and *Staphylococcus aureus* (A) *Persea americana* (Avocado), (B) *Pimenta dioica* (Allspice), (C) *Cinnamomum tamala* (Tejpatta) and (D) *Cinnamomum verum* (Dalchini).

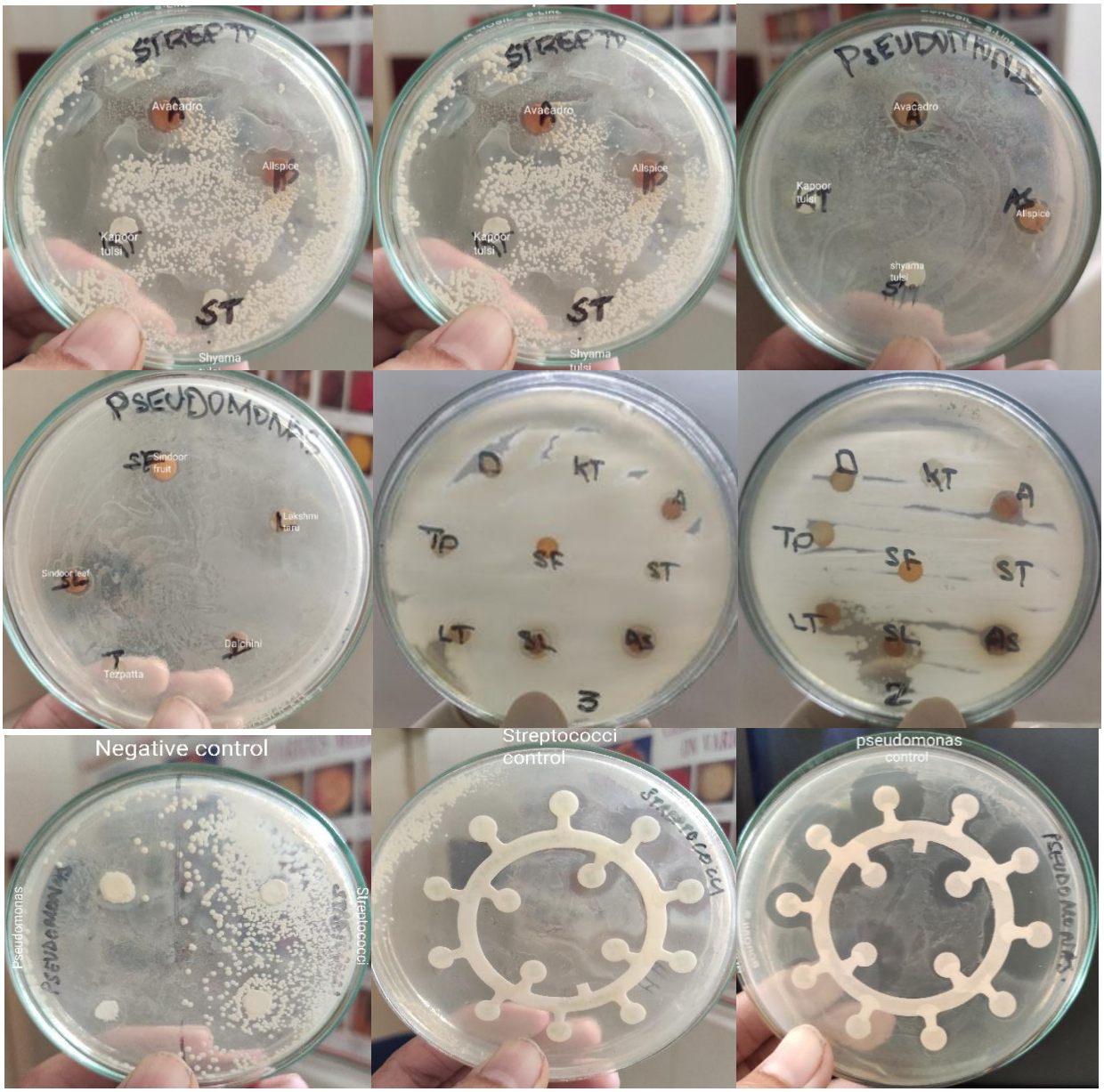

Figure 3: Zone of inhibition (mm) of different plant extracts against *Pseudomonas aeroginosa* and *Streptococcus* spp. by disc diffusion method.

DISCUSSION

Several researchers had analyzed antimicrobial activity on *Persea americana*, *Pimenta dioica*, *Cinnamomum tamala* and *Cinnamomum verum*. Our work includes the ethanolic extract of plants of above plants. In our study *P. americana* (Avocado) showed potent antimicrobial activity with a zone of inhibition of 7 mm, 8 mm and 12 mm against *Streptococci* spp., *P. aeruginosa*, and *S. aureus* respectively. The plant extract also inhibited the growth of bacteria in growth inhibition assay. Whereas, other researchers showed that methanolic extract of leaf gives highest zone of inhibition of 6.0 mm and for bark it gives the maximum zone of inhibition 12.0 mm against *S. aureus* using agar well diffusion method.[11]

Most of the scientists had worked on fruit extract of *P. americana* (Avocado) and showed its antimicrobial activity. In a study, organic extracts prepared from seeds of two avocado species showed adequate antibacterial activity against gram positive *S. aureus* showing a zone of inhibition 19.03 mm by disc diffusion method.[12] Another study on antibacterial activity of four varieties of avocado fruits was done by Amado and co-workers (2019) using *E. coli*, *S. aureus*, *Salmonella* and *B. cereus.* They found that peel and seed show less antimicrobial activity in gram negative bacteria than that of gram positive bacteria.[13] The plant also showed antibacterial activity against a strain *Porphyromonas gingivalis*, isolated from primary endodontic infection with minimum bactericidal concentration (MBC) value at a concentration of 60% and MIC value at a concentration of 50%.[14] The methanolic extracts of *P.*

americana showed potent antimicrobial activity against two MDR strains of *M. tuberculosis* H37Rvand H37Ra with MIC value of 62.5 µg/ml and 125 µg/ml respectively [15]. Another study showed antibacterial activity in methanolic extracts were more effective than acetone extract against clinical isolates which includes *Pseudomonas aeruginosa, Bacillus cereus, Salmonella typhi, Bacillus subtilis, Shigella flexneri, Escherichia coli* and *Staphylococci aureus.*[16]

The aqueous and ethanolic seed extracts of *P. americana* suppressed the growth of gram-positive and gram-negative bacteria. The aqueous extracts have activity against *Staphylococcus epidermidis* and *Listeria monocytogenes* with MIC values of 354.2µg/mL and 93.8 - 375.0 µg/mL respectively.[17] The antimicrobial potential of extracts of *P. dioica* (Allspice) were reported by several researchers and our study showed potent effect against *P. aeruginosa, E. coli, Streptococci* spp. and *S. aureus* with a zone of inhibition of 8 mm, 6 mm, 8 mm and 18 mm respectively. One study showed ethanolic leaves extract of *P. dioica* showed potent antibacterial activity against different bacterial strains in the order of *Staphylococcus aureus* < *Salmonella typhimurium* < *Bacillus cereus* < *Escherichia coli.*[18] Whereas, some researchers gave significant activity in hexanes and alcoholic extracts with 32.1 ± 0.26 mm and 20.3 ± 0.16 mm zone of inhibition against gram-positive *B. megaterium* and gram negative *P. fluorescens* respectively by disc diffusion method.[19] The methanolic extract of leaf and bark of Allspice was found highly effective against *S. mutans* than *S. aureus* recovered from burn and dental caries patients[20] Hari and co-workers (2010) studied antibacterial activity in essential oil and aqueous extract of *P. dioica* for against five different microbial species (including both gram negative and gram positive strains).[21] The essential oils of leaves of *P. dioica* also exhibited potent antimicrobial activity against *B. cereus, S. typhimurium* and *S. aureus* by disc diffusion method.[22] In another study MBC and MTC (maximally tolerated concentration) values of essential oil of *P. dioica* was obtained against *P. putida* was found to be 0.1% wt/vol and 0.05% wt/vol respectively.[23]

Cinnamomum tamala is known for its medicinal property and used as a spice in India. The presence of major phytochemical constituents detected in methanolic extracts of *C. tamala* were tannins, alkaloids, flavonoids, and terpenoids which were responsible for its antibacterial activity [24]. Another study showed acetone, methanol and ethanolic extract of leaves of Tejpatta showed inhibitory action against both *Bacillus cereus* and *Serratia*. The zone of inhibition of acetone extract against *B. cereus* was found 26.6 ± 0.57 mm by agar well diffusion method.[25] Whereas, the methanolic leaf extract of *C. tamala* showed activity in the sequence of *P. aeruginosa* < *K. pneumonia* < *E. coli* < *S. aureus.*[26] In another study, methanolic extract of leaves of *C. tamala* exhibited potent antimicrobial activity against clinical samples of 8 different multidrug-resistant Gram negative bacteria namely *Pseudomonas spp, K. pneumonia, Citrobacter spp., P. vulgaris, K. oxytoca, E. coli, Acinetobacter* spp. *and P. mirabilis* [27]. Even essential oil taken from dried and fresh leaves of *C. tamala* showed potent antibacterial activity against *M. luteus, B. subtilis, S. aureus,* and *P. aeruginosa* [28]. In present study, the ethanolic extracts of *C. tamala* and *C. verum* showed the growth inhibition of *P. aeruginosa* by growth inhibition assay whereas, both the extracts were found inactive when evaluated by disc diffusion method. Whereas other extracts showed moderate action against all the strains used for the study. The methanolic extracts of leaves and bark were tested against *P. aeruginosa, S. aureus, E. coli, B. cereus* by agar well diffusion method and agar dilution method and was found to be potential sources of antimicrobial agents [29]. The ethanolic extract of cinnamon bark exhibited an antibacterial effect against *K. pneumonia* and *E. coli* with zone of inhibition 25.50 ± 3.72 mm and 11.72 ± 1.86 mm respectively.[30] In another study ethanolic extract and essential oil of *C. verum* were studied by Hamedo (2015) found it effective against *E. coli* and *P. aeruginosa* [31]. Cinnamaldehyde and eugenol present in the essential oil of *C. verum* are responsible for the bactericidal activity and found effective against Paenibacillus larvae and gave MBC and MIC of 125-250µg/ml and 25-100µg/ml respectively by tube dilution method.[32] The ethanolic extracts of cinnamon bark were found potent against four bacterial strains namely *S. aureus, E. coli, S. mutans,* and *Peptococcus* isolated from orofacial infections [33]. The methanolic extract and its oil of *C. verum* also found to have a bacteriostatic action against *E. coli, P. aeruginosa,* and *B. subtilis* bacteria found in ketchup.[34]

CONCLUSION

The ethanolic extracts prepared from all the four plants showed potent antimicrobial activity against two gram negative and gram positive bacteria. The extract of *Cinnamomum tamala* showed the highest effect as compared to other extracts. The plant can be further studied and investigated. The investigation and research on medicinal plants might bring to the scientific world many useful remedies for the treatment and cure of human sufferings.

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