

Preliminary Study on the Antibacterial Activity of Six Medicinal Plants against Two Naso-Pharyngeal Pathogens—*Streptococcus pyogenes* and *Pseudomonas aeruginosa*

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Abstract

Objective: The objective is to study the antibacterial activity of six medicinal plants against two naso-pharyngeal pathogens and determination of total phenol contents in ethanol extracts of those plants. **Methods:** Different serial concentrations (0.05 g/mL, 0.1 g/mL, 0.2 g/mL, 0.4 g/mL) of ethanolic and acetone extracts of *Piper nigrum* L. (*Piperaceae*), *Ocimum sanctum* Linn., *Plectranthus amboinicus* L. (*Lamiaceae*), *Ayapana triplinervis* M.Vahl. (*Asteraceae*), *Cinnamomum zeylanicum* L. (*Lauraceae*), *Allium schoenoprasum* Linn. (*Liliaceae*) were evaluated for the antibacterial activity using disc diffusion method against gram positive *Streptococcus pyogenes* and gram negative *Pseudomonas aeruginosa*. The extracts were prepared from different parts of the plants. The total phenol content was estimated using folin-ciocaltau reagent in catechol equivalents. **Results:** Majority of the extracts had inhibitory effect against the tested bacteria at different concentrations. In ethanol extracts, *Plectranthus amboinicus* exhibited the maximum zone of inhibition (14 mm) at 0.05 g/mL concentration against *Streptococcus pyogenes*, and *Ocimum sanctum* showed highest zone of bacterial inhibition (19 mm) at 0.05 g concentration against *Pseudomonas aeruginosa*. In acetone extracts, *Piper nigrum* had the maximum zone of bacterial inhibition (17 mm) in 0.4 g/mL concentration against *Streptococcus pyogenes* and *Cinnamomum zeylanicum* and *Allium schoenoprasum* exhibited the highest zone of bacterial inhibition (0.4 g/mL) against *Pseudomonas aeruginosa*. The ethanol extract of *Plectranthus amboinicus* contained the highest amount of phenol (0.8 mg/mL) and *Allium schoenoprasum* contained the lowest amount (0.62 mg/mL). In acetone, *Cinnamomum zeylanicum* contained highest phenol content (0.78 mg/mL). **Conclusion:** All these investigations pave way to the molecular modeling of the lead phyto compounds present in the

studied plants, and also in finding out their biochemical action in various metabolic pathways and reactions of infection.

Keywords

Multiple Resistance, Human Pharyngitis, Phytochemicals, Antibiotics, Beta-Haemolytic Bacteria, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Natural Products

1. Introduction

The increase in the number of multi-drug resistant microbial strains (MDR phenotypes) and the appearance of strains with reduced susceptibility to antibiotics is proved to be the major concerns all around the globe. Among the human pathogenic bacteria, gram negatives are the most vulnerable [1]. Resistance may be innate or acquired. Most commonly antimicrobial resistance refers to acquired resistance which may be a result of either novel mutation or transfer of gene causing resistance. The use of plant derivatives as antimicrobials has not been extensively addressed until recently since most antibiotics were derived from microbes. Effective lifespan of any antibiotic is limited and the increased resistance shown by microbes leads to the investigation of new sources such as phytochemicals that are heavily investigated today [2]. The local use of natural plants as primary health remedies, due to their pharmacological properties and cheap availability, is quite common in Asia, Latin America and Africa. A large proportion of phytochemicals are currently used as lead molecules in drug discovery to produce synthetic molecular analogs. There are many evidences of using plant extracts for the treatment of many microbial diseases in traditional medical system.

Pharyngitis is a common problem of any infection related with the respiratory system. Patients of almost all age groups are being affected by this disease more than twice in a year, most frequent in children. Bacteria are responsible for approximately 10% of the pharyngitis infection, with group A beta-haemolytic *Streptococci* being the most common bacterial etiology [3]. Antibiotic therapy with Amoxicillin-Clavulanate or Azithromycin on nasopharyngeal colonization of *Streptococcus pneumoniae* and *Haemophilus influenzae* in children does not directly increase the number of resistant strains in the population, but by eradicating susceptible strains, it allows greater opportunity for carriage and spread of resistant strains [3]. Even though, Amoxicillin-Clavulanate potassium is an effective antibiotic, many group “A” strains show resistance to this antibiotic [4]. Azithromycin/zithromax and Amoxyllin do not break down in the body as quickly as other antibiotics. Instead of floating freely in the blood, it gets absorbed by WBC that fights bacteria. The WBC takes the medicine to the frontlines of their struggles with germs, where it becomes concentrated in the tissues surrounding the infection. Side effects found in 12% of patients, among them about 10% are severe. Patients with low levels of K and Mg in blood and lower heart beats can lead to abnormal changes in the electric activity of heart leading to potentially fatal irregular heart rhythm. There are the side effects noticed with Amoxyllin and Azithromycin antibiotic treatments [5].

Many investigations were carried out all around the globe for the radical scavenging activities of many phyto-constituents. Phytochemicals boost the host’s anti-inflammatory defense and sensitize malignant cells to cytotoxic agents [6]. Future pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from medicinal plants are crucial.

In recent times, plant researches have increased all over the world and a large number of evidence has been collected to show immense potential of medicinal plants used in various traditional system. Chemical antibiotics are not the last word for the treatment because of the increased number of MDR phenotypes of human pathogens. In view of developing potential herbal drugs, we need to understand the effectiveness of phytochemicals in many plants. No literature was available for the comparative study of the antibacterial activity of a group of medicinal plants extracts against two particular bacteria. In order to understand the potential antibacterial activity of phytochemicals in six plants, we designed this experiment. In this study, we selected plants based on their importance in the traditional Indian Folklore Medicine, for the treatment of infections related with pharynx.

2. Materials and Methods

2.1. Plant Material

The plant materials selected for the study are *Piper nigrum* L. (*Piperaceae*), *Ocimum sanctum* Linn., *Plectranthus amboinicus* L. (*Lamiaceae*), *Ayapana triplinervis* M.Vahl. (*Asteraceae*), *Cinnamomum zeylanicum* L. (*Lauraceae*), *Allium schoenoprasum* Linn. (*Liliaceae*). They were collected from the Botanical garden of NSS College, Pandalam, Kerala, in March 2015. The taxonomic identification was done comparing the existing herbarium in Botany Department of Kerala University and in the laboratory using standard keys [7].

2.2. Extract Preparation

For extract preparation different parts of the plants were selected. Leaves of *Ayapana triplinervis*, *Plectranthus amboinicus* and *Ocimum sanctum*, stem of *Piper nigrum*, bark of *Cinnamomum zeylanicum* and bulbil of *Allium schoenoprasum* were used for drying. The plant parts were dried in an oven at 40°C and then powdered using an electronic blender. The ethanolic and acetone extracts were prepared using 1g of each plant powder and 10ml of 80% ethanol and acetone (ethanol/acetone-distilled water; 8:2 w/v). After that the samples were centrifuged (3000 rpm) for 15 minutes, and their supernatants were harvested. This procedure was repeated three times. Eventually, the extracts were placed at room temperature in order to solvent evaporation [8].

2.3. Bacterial Species

The two bacterial species used in this investigation were originally clinical isolates from patients. One gram-positive species *Streptococcus pyogenes* (*S. pyogenes*) and one gram negative species *Pseudomonas aeruginosa* (*P. aeruginosa*). They were identified according to standard phenotypic tests.

2.4. Determination of Antibacterial Activity

Four concentrations of each extract (0.05 g/mL, 0.1 g/mL, 0.2 g/mL, and 0.4 g/mL) were prepared and their antibacterial activity was assessed by disc diffusion method against the test Bacteria. Stock culture of test bacteria were grown in nutrient broth medium at 37°C for 22 hr. Final bacterial number was adjusted to 0.5 Mc Farland turbidimetry [9]. Sterile 6mm filter paper discs were placed in the different concentrations of plant extract for 2 hours in order to solvent absorption. 20 ml each of sterile Muller Hinton Agar (MHA, Merck) medium were poured into each pre-sterilized and pre-labelled petriplates. A lawn culture then prepared on MHA using sterile cotton swab for each Bacteria in separate petriplates. Sterile filter paper discs were placed on these cultures and impregnated with 50 µL of each concentration. A total of 12 petriplates containing MHA were prepared for 6 plants with two bacteria. One bacterium was inoculated on 6 petriplates and the other one on the other 6 petriplates. The plates were left at room temperature for about 1 h to allow the extract to diffuse from the discs into the medium, and were then incubated at 37°C for 24 hrs. After incubation, the diameter zone of bacterial growth inhibition around each disc was measured with transparent ruler and recorded in millimeter. Standard antibiotics including Tetracycline (TE) 30 mcg, Azithromycin (AZK) 15 mcg, and Amoxycillin (AX) 10 mcg were used as controls for comparing the results. In order to determine the possible inhibitory effect of acetone and ethanol on the test bacteria, discs containing 80% acetone and ethanol were also tested. All the experiments were repeated in triplicate and the averages were taken as the result. All the results were recorded and tabulated in separate books. The photographs of culture plates, extracts, and media were taken using a digital camera (Sony, 13 mp).

2.5. Determination of Total Phenol Content

Total phenol content in different plant extracts in ethanol and acetone were determined by spectrophotometer using Folin-ciocaltueau reagent as milligram per micro Litre catechol equivalents [10]. The solution mixture was allowed to stand for 15 minutes at room temperature and the absorbance was measured at 750 nm.

3. Results

The results of antimicrobial activities of the ethanolic and acetone extracts of these plants are presented in **Table 1** and **Table 2**. This antibacterial activity was quantitatively determined by the presence or absence of inhibition zone around the discs containing extracts.

Table 1. Inhibition zone (mm)* of 6 selected medicinal plants-ethanol extract at various concentrations on tested bacteria.

Bacterial sp.	<i>Allium schoenoprasum</i> (g/ml)				<i>Cinnamomum zeylanicum</i> (g/ml)				<i>Plectranthus amboinicus</i> (g/ml)				<i>Ayapana triplinervis</i> (g/ml)				<i>Ocimum sanctum</i> (g/ml)				<i>Piper nigrum</i> (g/ml)			
	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4
<i>Strept. pyogenes</i> (Gram +)	10	9	13	8	13	11	10	12	14	11	8	10	13	9	8	10	12	6	8	13	9	10	R	R
<i>Pseudom. aeruginosa</i> (Gram -ve)	10	15	R	R	17	11	6	7	R	7	R	R	R	8	5	6	19	7	8	4	5	7	12	8

R: Resistant, * (6 mm) diameter disc.

Table 2. Inhibition zone (mm)* of 6 selected medicinal plant-acetone extract at various concentrations on tested bacteria.

Bacterial sp.	<i>Allium schoenoprasum</i> (g/ml)				<i>Cinnamomum zeylanicum</i> (g/ml)				<i>Plectranthus amboinicus</i> (g/ml)				<i>Ayapana triplinervis</i> (g/ml)				<i>Ocimum sanctum</i> (g/ml)				<i>Piper nigrum</i> (g/ml)			
	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4
<i>Strept. pyogenes</i> (Gram +)	8	14	6	9	5	6	5	9	R	10	6	7	13	R	14	10	10	9	10	10	10	16	10	17
<i>Pseudom. aeruginosa</i> (Gram -ve)	2	5	9	10	3	4	5	10	6	9	7	9	R	5	3	4	5	6	R	7	8	6	2	9

R: Resistant, * (6 mm) diameter disc.

3.1. Antibacterial Activity of Different Plant Extracts Prepared in Ethanol against the Test Bacteria in Comparison with Control

All extracts showed good responses. For *S. pyogenes* the highest Zone of Inhibition (ZI) was noticed in the extracts of *Plectranthes amboinicus* (14 mm) at 0.05 g/mL concentration, and the bacterium showed resistance against two concentrations (0.2 & 0.4 g/mL) of *Piper nigrum* extract, where no visible zone of inhibition present. *Plectranthes amboinicus* is considered as the most effective plant among the 6 studied against Streptococcal infection. The highest ZI for *Allium schoenoprasum* was at 0.2 g/mL concentration (13 mm). The highest ZI (13 mm) for *Cinnamomum zeylanicum* and *Ayapana triplinervis* were noticed at the concentration 0.05 g/mL respectively. For *Ocimum sanctum* also the highest ZI (13 mm) was the same as that of *Cinnamomum* and *Ayapana*, but it was at the concentration 0.4 g/mL. For all the other concentrations of all the 6 plants studied, the ZI varied between 6 and 10 mm for this bacterium.

The highest zone of inhibition against the gram negative *Pseudomonas aeruginosa* was in the extracts of *Ocimum sanctum* (19 mm) at the lowest concentration of 0.05 g/mL. The second highest against this bacterium was noticed in *Cinnamomum zeylanicum* (17 mm) at the lowest concentration of 0.05 g/mL as that of *Ocimum*. The next highest value was noticed in *Allium shoenoprasum* (15mm at 0.1 g/mL), and in *Piper nigrum* 12mm at 0.2 g/mL. *Plectranthus amboinicus* and *Ayapana triplinervis* was least effective against this bacterium with a ZI of 7 and 8 mm respectively at 0.1 g/mL. For all the other concentrations of all the 6 plants studied, the ZI varied between 4 and 10 mm for this bacterium. Resistance was shown against 0.2 and 0.4 g/mL of *Allium*, whole concentrations of *Plectranthus* except 0.1 g/mL, and 0.05 g/mL of *Ayapana*.

3.2. Antibacterial Activity of Different Plant Extracts Prepared in Acetone against the Test Bacteria in Comparison with Control

For *Streptococcus pyogenes* the highest ZI (17 mm) was found at 0.4 g/mL concentration of *Piper nigrum*, the second highest value (16 mm) was also observed for the same extract at 0.1 g/mL concentration. In *Cinnamomum zeylanicum* extract bacteria showed least ZI (5 mm) at 0.05 g/mL concentration. In *Plectranthus amboini-*

cus and *Ayapana triplinervis* extracts, *S. pyogenes* showed resistance at concentrations 0.05 and 0.1 g/mL. The ZI for other plant extracts ranged between 6 to 14 mm. The highest ZI against the gram negative *Pseudomonas aeruginosa* was noticed in the extracts of *Cinnamomum zeylanicum* (10 mm) and *Allium schoenoprasum* (10 mm) at 0.4 g/mL. The lowest ZI (2 mm) was noticed in both *Allium schoenoprasum* and *Piper nigrum* at 0.05 and 0.2 g/mL concentrations respectively. *Ocimum sanctum* and *Ayapana triplinervis* showed resistance at 0.2 g/mL and 0.05 g/mL respectively. *Ocimum sanctum* showed highest zone of inhibition (7 mm) at 0.4 g/mL concentrations. *Plectranthus amboinicus* showed highest zone of inhibitions (9 mm) at two concentrations (0.1 and 0.4 g/mL).

Ethanol and acetone could not be a factor that might affect these results, because the discs with 80% ethanol and acetone not showed much inhibitory effect on the test bacteria (Table 3) while it is compared to the plant extracts in combination with these solvents. It may be due to the volatile nature of ethanol and acetone. But the antibiotics AX, AZK, and TE showed significant results against the test bacteria compared to ethanol and acetone. Azithromycin (ZI against *S. pyogenes* was 14mm and *P. aeruginosa* was 25 mm) was significantly more effective against both the bacteria compared to Amoxycilin and Tetracycline (Table 4).

3.3. Total Phenol Content of Plant Extracts

In ethanol extract, the amount of total phenol is highest in *Plectranthus amboinicus* (0.8 mg/mL). The second highest was noticed in *Cinnamomum zeylanicum* (0.78 mg/mL). The phenol content in *Piper nigrum*, *Ayapana triplinervis* and *Ocimum sanctum* were 0.74 m g/mL, 0.68 m g/mL and 0.64 m g/mL respectively. *Allium schoenoprasum* had least amount of phenol content (0.62 mg/mL). In acetone extract, the amount of total phenol was highest in *Cinnamomum zeylanicum* (0.78 mg/mL). The second highest was found in *Plectranthus amboinicus* (0.77 mg/mL). In *Allium schoenoprasum*, *Piper nigrum* and *Ocimum sanctum* the content of total phenol was 0.74 m g/mL, 0.69 m g/mL and 0.66 m g/mL respectively. *Ayapana triplinervis* had the least amount of phenol (0.63 mg/mL) content (Table 5).

Table 3. Inhibition zone (mm), in different concentrations of ethanol and acetone against the tested bacteria.

Bacterial Sp.	Different concentrations of ethanol (g/mL)				Different concentrations of acetone (g/mL)			
	0.05	0.1	0.2	0.4	0.05	0.1	0.2	0.4
<i>S. pyogenes</i>	R	R	R	R	5	R	R	R
<i>P. aeruginosa</i>	6	6	5	5	5	R	8	5

*values in mm, R—resistant.

Table 4. Growth of bacteria in control.

Sl. No.	Name of Bacteria	Amoxicillin (AX)*	Azithromycin (AZK)*	Tetracycline (TE)*
1	<i>Streptococcus pyogenes</i>	8	14	7
2	<i>Pseudomonas aeruginosa</i>	8	25	13

*zone of inhibition against antibiotics in mm.

Table 5. Total phenol content of different plant extracts.

Sl. no.	Plants	Phenolic content in ethanol	Phenolic content in acetone
		µg catechol equivalents	µg catechol equivalents
1	<i>Allium schoenoprasum</i> L.	0.62 mg/mL	0.74 mg/mL
2	<i>Ayapana triplinervis</i> M.Vahl	0.68 mg/mL	0.63 mg/mL
3	<i>Cinnamomum zeylanicum</i> L.	0.78 mg/mL	0.78 mg/mL
4	<i>Ocimum sanctum</i> L.	0.64 mg/mL	0.66 mg/mL
5	<i>Piper nigrum</i> L.	0.74 mg/mL	0.69 mg/mL
6	<i>Plectranthus amboinicus</i> Spreng.	0.8 mg/mL	0.77 mg/mL

Values are mean of 3 experiments.

4. Discussion

Plant extracts has been used in traditional medicine to treat bacterial diseases since time immemorial. Traditionally used medicinal plants produce a variety of compounds with known therapeutic properties. Plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of gastroenteritis, uveitis, otitis media, typhoid fever and wound infections. The plant derived substances can either inhibit the growth or kill the pathogen or have no or least toxicity to the host cells. These should be considered as important for developing new antimicrobial drugs.

4.1. Antibacterial Activity of Different Plant Extracts in Ethanol and Acetone against *Streptococcus pyogenes*

In the ethanolic extract for *S. pyogenes* the highest ZI (14 mm) was at 0.05 g/mL concentration of *Plectranthes amboinicus*, and all the different concentrations of this plant extract showed very good antibacterial activity compared to all other plant extracts (Figure 1). While the acetone extracts of this plant was less effective and the bacterium showed resistance. *Plectranthes amboinicus* in ethanol was the most effective plant extract among the 6 plants studied against Streptococcal infection as per this study. This plant is used against pharyngitis in traditional system of medicine for a long period. It is reported to contain many essential oils having the property of anti biofilm formation in many bacteria [11]. Phenolic compounds present in berries selectively inhibit the growth of human gastrointestinal pathogens. Phenolics inhibit the adherence of bacteria to epithelial surfaces and further prevent colonization and infection [12].

But in the acetone extract the highest ZI (17 mm) was found at 0.4 g/mL concentration of *Piper nigrum*, and the second highest value (16 mm) was also observed for the same extract at 0.1 g/mL concentration (Figure 2). In the other two concentrations studied, the bacterium showed resistance. Many of the research works support the efficacy of *Piper nigrum* against this bacterium. *Piper nigrum* DMS extract showed good antibacterial effect with high ZI (10 mm) [13]. Pepper-ethanol extract was ineffective and methanol extract of this plant was very effective [14]. *Piper nigrum* has well anti biofilm properties. It is a natural spice widely used in the Ayurvedic medicine to treat asthma, cough, diabetes and heart problems. Chloroform and petroleum ether extracts of *Piper nigrum* showed good inhibition against the bacteria at 2 mg/mL. While ethanol and ethyl acetate extracts of *P. nigrum* didn't show significant results [15].

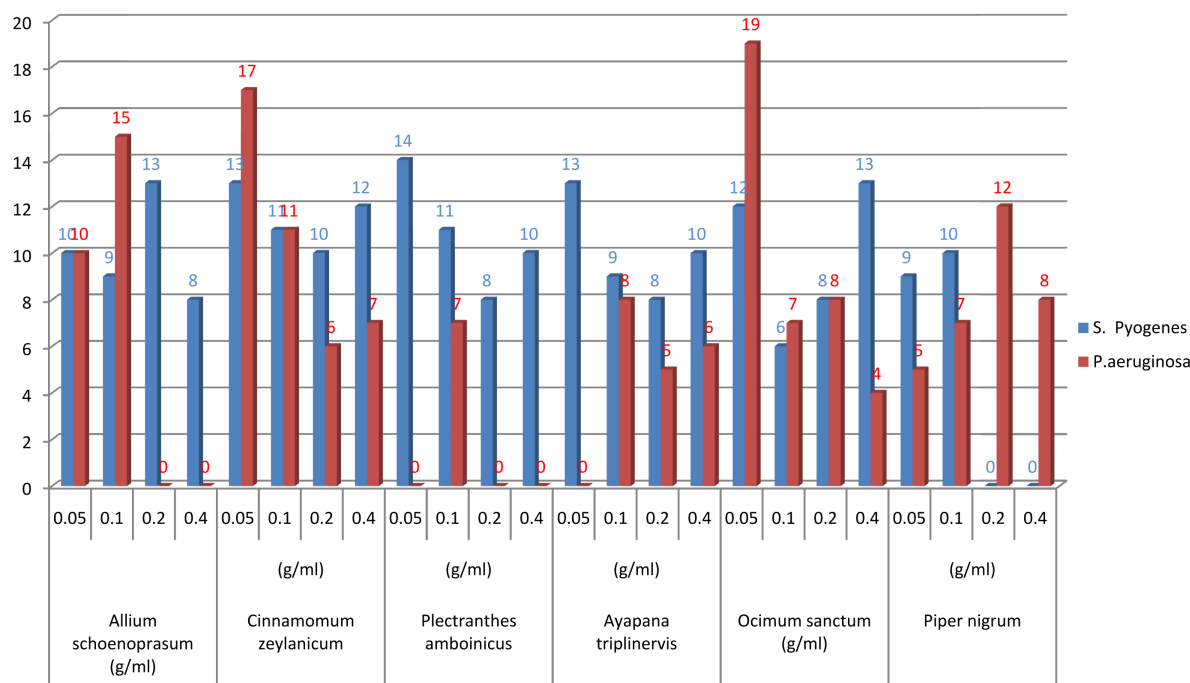


Figure 1. Zone of inhibition of six medicinal plants extracts (4 different concentrations) in ethanol. Values are mean of three experiments in each group.

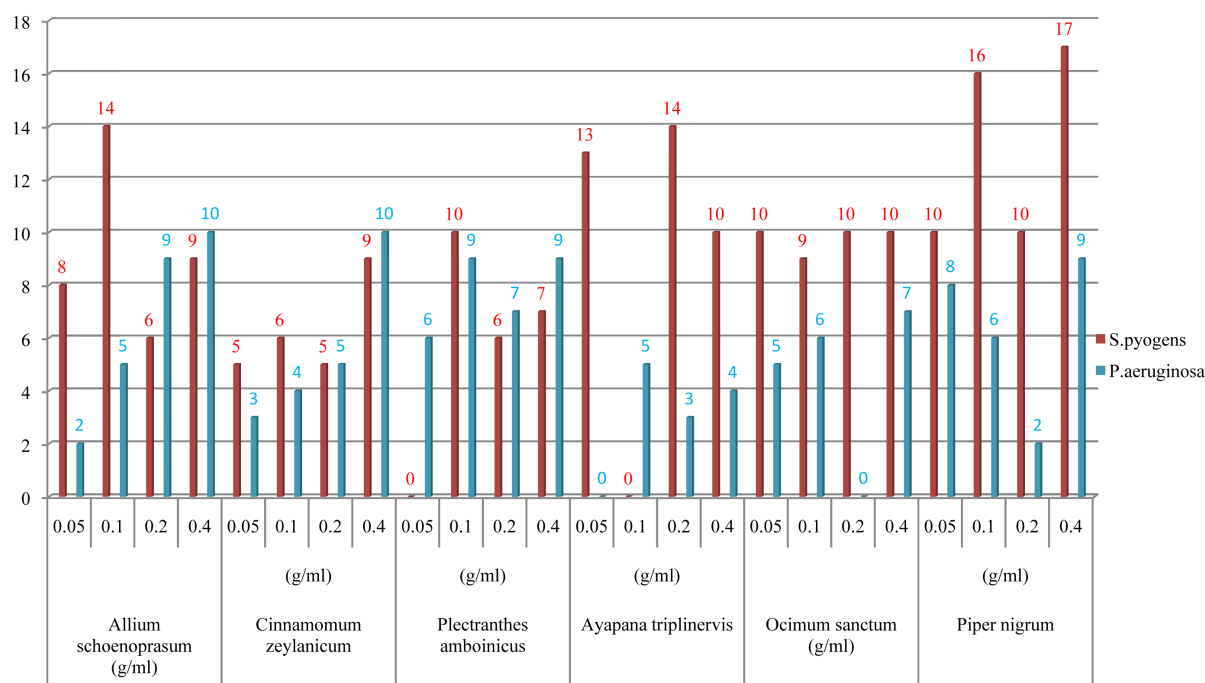


Figure 2. Zone of inhibition of six medicinal plants extracts (4 different concentrations) in acetone. Values are mean of three experiments in each group.

The ethanolic and acetone extracts of *Cinnamomum zeylanicum*, *Ayapana triplinervis*, *Ocimum sanctum*, *Allium schoenoprasum* were also very effective against *S. pyogenes*. Among them *Ocimum* showed good responses in all the tested concentrations.

4.2. Antibacterial Activity of Different Plant Extracts in Ethanol and Acetone against *Pseudomonas aeruginosa*

Ocimum sanctum had the highest zone of bacterial inhibition against *Pseudomonas aeruginosa* in ethanol extract. It contains a number of phytochemicals, essential oils, phenolic compounds and other natural products including polyphenols such as flavanoids and anthocyanin etc. It is an important component in Ayurvedic treatment of many diseases, and has anti fertility, anticancer, anti diabetic, antifungal and antibacterial properties. Ursolic acid, oleanolic acid and betulinic acid present in *Ocimum* are bioactive compounds of antibacterial activity against bacterial species [16]. The phenolic compounds and flavanoids are potent antioxidants, free radical scavengers and metal chelators. *Ocimum sanctum* is a better alternative as a preservative in food industries since it is equally effective against pathogenic gram positive and negative bacteria. *Ocimum*-chloroform extract showed maximum antibacterial activity against the growth of *P. aeruginosa* [17]. *Ocimum sanctum* extract can prevent AHL-mediated bacterial infection in higher organisms [18]. Methanol extract of *Cinnamomum* showed significant antibacterial effect (9 - 15 mm) against *P. aeruginosa*. Combination of plant and antibiotic extracts of amoxyllin, erythromycin and ciprofloxacin did not show significant activity compared to the plant extract alone [19]. The acetone extracts of Clove at 30 micro litre concentrations showed ZI-7mm for *P. aeruginosa*. Antibacterial activity of different plant varies according to phytochemical differences and collection sites. Among the plants studied, Clove found to have the most antibacterial activity [14].

4.3. Total Phenol Content of Plant Extracts

Plectranthes amboinicus was found to have the highest amount of total phenols (0.8 mg/mL) among all the six plants studied and this plant was the most effective against *Streptococcus pyogenes* as per this study. The plant extract in ethanol was found to be more effective because the amount of total phenol content was more in ethanol-*Plectranthus* extract than in acetone-*Plectranthus* extract (Figure 3 and Figure 4). The second highest

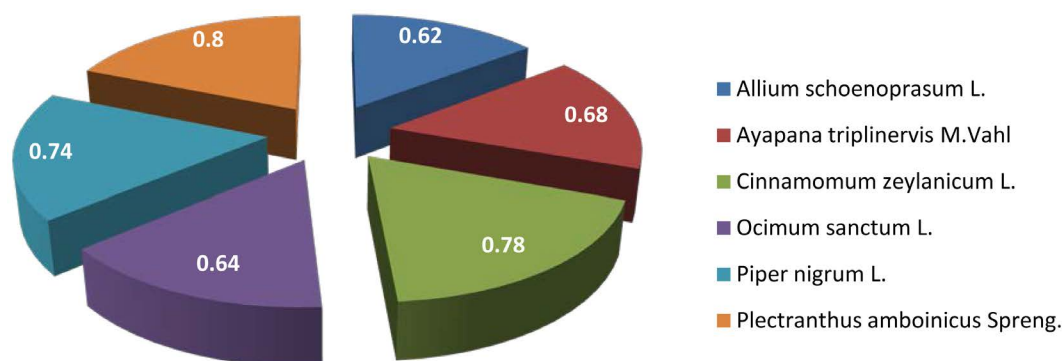


Figure 3. Phenol content of six plants in ethanol (µg catechol equivalents).

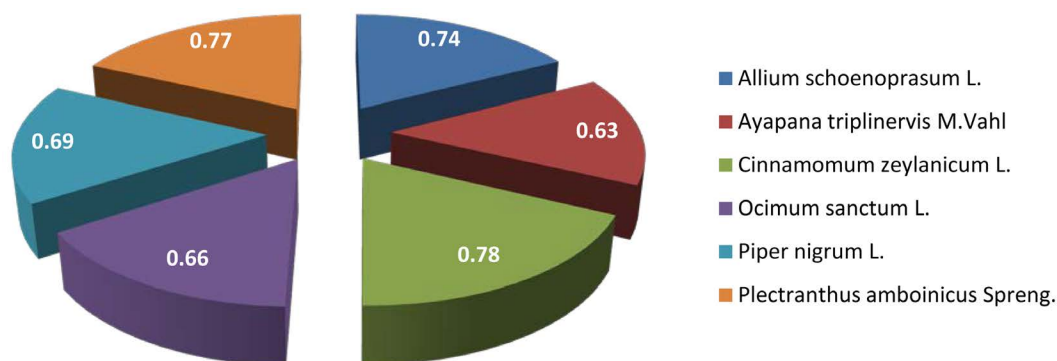


Figure 4. Phenol content of six plants in acetone (µg catechol equivalents).

amount of total phenols was found in *Cinnamomum zeylanicum* (in ethanol and acetone 0.78 mg/mL) and the extract of this plant was found most effective against the gram negative *Pseudomonas aeruginosa*. Dimethyl sulphoxide activity of *Cinnamon* oil was studied against *P. aeruginosa* and this organism was proved to be the most resistant with only 13 of 51 oils showing any inhibitory activity [11].

Antibacterial activity of different plants varies according to the phytochemical differences and the interaction with the bacterial cell. Eliciting such biochemical action is a future challenge in this regard. Careless management of the precious plant resources needs to be stopped and more investigations should be carried out to understand the less explored plants considered as weeds, to understand their valuable antimicrobial activities. Moreover, strict decision should be taken to understand the traditional herbal practices and to conserve such knowledge for the well being of the human society in the future because more and more multiple drug resistant pathogens are arising day by day.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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References

- [1] Koochak, H., Seyyendnejad, S.M. and Motamedi, H. (2010) Preliminary Study on the Antibacterial Activity of Some Medicinal Plants of Khuzestan (Iran). *Asian Pacific Journal of Tropical Medicine*, **3**, 180-184. [http://dx.doi.org/10.1016/S1995-7645\(10\)60004-1](http://dx.doi.org/10.1016/S1995-7645(10)60004-1)
- [2] Akrayi, H.F.S. and Abdulrahman, Z.F.A. (2013) Evaluation of the Antibacterial Efficacy and the Phytochemical Anal-

- ysis of Some Plant Extracts against Human Pathogenic Bacteria. *Journal of Pharmacy and Clinical Sciences*, **7**, 29-39.
- [3] Ghaffar, F., Muniz, L.S., Katz, K., Reynolds, J., Smith, J.L., Davis, P., Friedland, I.R. and McCracken Jr., G.H. (2000) Effects of Amoxicillin/Clavulanate or Azithromycin on Nasopharyngeal carriage of *Streptococcus pneumonia* and *Haemophilus influenza* in Children with Acute Otitis Media. *Clinical Infectious Diseases*, **31**, 875-880. <http://dx.doi.org/10.1086/318160>
 - [4] Hayes, C.S. and Williamson, H. (2001) Management of Group A Beta-Hemolytic *Streptococcal* Pharyngitis. *American Family Physician*, **63**, 1557-1564.
 - [5] (2012) United States Food and Drug Administration Report (FDA).
 - [6] Doughari, J.H., Elmahmood, A.M. and Mansara, S. (2007) Studies on the Antibacterial Activity of Root Extracts of *Carica papaya* L. *African J of Microbiology Research*, **1**, 037-041.
 - [7] Gamble, J.S. (1984) Flora of the Presidency of Madras. Vol. 1, 2, 3, Newman and Adlard Publishers, London.
 - [8] Aneja, K.R. (2003) Experiments in Microbiology and Plant Pathology. New Age International Pvt. Ltd., New Delhi.
 - [9] Burt, S.A. and Reinders, R.D. (2003) Antibacterial Activity of Selected Plant Essential Oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology*, **36**, 162-167. <http://dx.doi.org/10.1046/j.1472-765X.2003.01285.x>
 - [10] Gautam, M.K., Gangwar, M., Nath, G., Rao, C.V. and Goel, R.K. (2012) *In Vitro* Antibacterial Activity on Human Pathogens and Total Phenolic, Flavanoid Contents of *Murray paniculata* Linn. Leaves. *Asian Pacific Journal of Tropical Medicine*, S1660-S1663.
 - [11] Hili, P., Evans, C.S. and Veness, R.G. (1997) Antimicrobial Action of Essential Oils: The Effect of Dimethyl Sulphoxide on the Activity of Cinnamon Oil. *Letters in Applied Microbiology*, **24**, 269-275. <http://dx.doi.org/10.1046/j.1472-765X.1997.00073.x>
 - [12] Puupponen-Pimia, R., Nohynek, L., Alakomi, H.-L. and Oksman-Caldentey, K.-M. (2005) The Action of Berry Phenolics against Human Intestinal Pathogen. *Biofactors*, **23**, 243-251. <http://dx.doi.org/10.1002/biof.5520230410>
 - [13] Limsuwan, S. and Voravuthikunchai, S.P. (2013) Anti *Streptococcus pyogenes* Activity of Selected Medicinal Plant Extracts Used in Thai Traditional Medicine. *Tropical Journal of Pharmaceutical Research*, **12**, 535-540. <http://dx.doi.org/10.4314/tjpr.v12i4.14>
 - [14] Keshkin, D. and Toroglu, S. (2011) Studies on Antimicrobial Activities of Solvent Extracts of Different Spices. *Journal of Environmental Biology*, **32**, 251-256.
 - [15] Priyadarsini, D.T., Srinivasan, P., Guna, G. and Manimekalai, K. (2015) *In Vitro* Anti Biofilm Activity of *Piper longum* and *Piper nigrum* against Clinical Isolates of *Streptococcus pyogenes* Isolated from Pharyngitis Patients. *International Research Journal of Pharmacy*, **6**, 122-132.
 - [16] Ali, R., Chauhan, V., Farooq, S., Khan, A. and Farooq, U. (2014) *In Vitro* Analysis of Antibacterial Activity of *Ocimum sanctum* against Pathogenic Bacteria and Quantification of Ursolic Acid and Aleanolic Acid. *International Journal of Pharmaceutical Sciences Review and Research*, **25**, 13-17.
 - [17] Mishra, P. and Mishra, S. (2011) Study of Antibacterial Activity of *Ocimum Sanctum* Extract against Gram-Positive and Gram Negative Bacteria. *American Journal of Food Technology*, **6**, 336-341. <http://dx.doi.org/10.3923/ajft.2011.336.341>
 - [18] Musthafa, S.K., Veera Ravi, A., Annapoorani, A. and Sybiya Vasantha Packiavathy, I. (2010) Evaluation of Anti-Quorum-Sensing Activity of Edible Plants and Fruits through Inhibition of the N-acetyl-homoserine Lactone System in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. *Journal of Chemotherapy*, **56**, 333-339. <http://dx.doi.org/10.1159/000320185>
 - [19] Vyas, P., Suthar, A., Patel, D., Dayma, P., Raval, J. and Joshi, D. (2015) Antimicrobial Activity of Extracts of *Cinnamomum zeylanicum* Bark and Its Combination with Antibiotics against Various Micro Organisms. *Journal of chemical and Pharmaceutical Res*, **7**, 68-70.