

Antimicrobial Activity of *Chromolaena odorata* Against Selected Pyogenic Pathogens

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ABSTRACT

A study of antimicrobial activity of the solvent extracts of *Chromolaena odorata* against the selected pyogens such as *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Candida albicans*. The plant materials were organoleptically evaluated and pulverized. Using petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water the plant material was subjected to Successive Solvent Extraction method. These extracts were screened for phytochemical components and tested for antimicrobial properties against the pyogens. The efficacy of the plant extracts was compared with the commercial antibiotics. When comparing all the extracts of *C.odorata*, the ethanolic extract showed more efficacy than the other extracts.

Keywords: *C. odorata*, Antimicrobial activity, Pyogenic infection, Solvent Extraction, Organoleptic evaluation.

INTRODUCTION

Pyogenic infection is an infection characterized by severe local inflammation, usually with pus formation, generally caused by the pyogenic microorganism. Humans are the natural host for many bacterial species that colonize the skin as normal flora. Skin infections are common and may be caused by bacteria, fungi or viruses. Human infection, particularly those involving the skin and mucosal surfaces may lead to serious complications. The most common bacteria which cause wound infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*¹.

S. epidermidis is an endemic microorganism, consisting of non-motile Gram-positive cocci, arranged in grape like clusters. The ability to form biofilms on plastic devices is a major virulence factor for *S. epidermidis*. It is part of the skin flora, and consequence part of human flora. It can also be found in the mucous membrane of humans and in animals². *Streptococcus pyogenes*, or Group A Streptococcus, is a spherical, Gram-positive bacterium. *S. pyogenes* became the reason of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. The most striking sign is a strawberry-like rash. *S. pyogenes* causes variety of infections of suppurative infections of the skin, including wounds, burns, with a predilection to produce lymphangitis and cellulitis. Infections of minor abrasions may leads to fatal septicemia³. *Pseudomonas aeruginosa* is a common bacterium which can cause disease in animals and humans. It is a slender gram negative bacillus, aerobic, rod-shaped bacteria with unipolar motility. *Candida albicans* is a diploid fungus and a causative agent of

opportunistic oral and genital infections in human. Systemic fungal infection in human has emerged as important cases of morbidity and mortality in immuno compromised patients. *Candida albicans* forms biofilm readily on the surface of implantable medical device. In addition to the intrinsic biological interest of the dimorphism, its ability to switch begins the yeast and the hyphal mode of growth has been implicated in its pathogenicity².

Traditional medicinal plants are used for the treatment of wound infection without any hazardous effects in body. Medicinal plants are widely used to cure different infection and also used as a precursor for the synthesis of natural drugs. It is reported that 20 medicinal plants from hazara division have antimicrobial activity against some selective organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli*⁴. *Chromolaena odorata* belongs to the family of Asteraceae. It is used as a traditional medicinal plant to cure wounds, cuts, burns, soft tissue aberrations. It has wound healing property and also promotes blood coagulation. It is used to treat diabetes and effective against the diarrhea strains⁵. The main aim of the study is to determine the efficacy of the plant extract *Chromolaena odorata* against the Pyogenic pathogen.

MATERIALS AND METHOD

Collection of pyogenic pathogens

The Pyogenic microorganisms *Staphylococcus epidermidis* (MTCC 3068), *Streptococcus pyogenes* (MTCC 14289) *Pseudomonas aeruginosa* (MTCC 741) and *Candida albicans* (MTCC 183) were procured from Microbial type culture collection centre. These organisms

Table 1: Organoleptic evaluation of *C. odorata*.

Parameters	<i>C. odorata</i>
Colour	Green
Odour	Raw, Pungent when crushed
Appearance	Dark green when powdered
Taste	Bitter

were subcultured on to their selective media.

Collection and Storage of Plant Materials

The leaves of *C. odorata* were collected from Shoarnur, Palakkad. The plant was authenticated in Botanical Survey of India (Authentication Number: BSI/SRC/5/23/2016/Tech/113). It was washed in running tap water to remove dust particles and subjected to shade drying for about one week. Dried leaves were pulverized with an electric mill. The sample were stored in an airtight container and tested for certain biologically active compounds.

Organoleptic evaluation of *C. odorata*

The plant parts were organoleptically evaluated and examined for various sensory parameters like colour, appearance of the plant parts mainly size and shape, external texture, fracture (granular, splintery, smooth) and external markings (furrow, wrinkles, ridges, annular, out growth) of the plant parts. The fragrance, test for odour (aromatic, balsamic, camphoraceous, spicy, pleasant, irritating) and taste (sweet, bitter, sour, astringent, pungent, acidic, alkaline) were evaluated^{6,7}.

Successive Solvent Extraction of plant materials

The phytochemical constituents were extracted using successive solvent extraction method based on polarity. About 20 grams of powdered leaves of *C. odorata* was separately mixed with 100mL of solvent (Petroleum ether, Benzene, Chloroform, Ethyl acetate, Ethanol, Water) and subjected to occasional shaking for 24h. The plant extracts were filtered using Whatmann No.1 filter paper. The filtrate was concentrated by evaporation at room temperature. During all the extraction with the next solvent, the residue was air dried thoroughly to remove the trace of solvent used^{8,9}.

Screening for Phytochemical Constituents

The dried plant extracts were screened for the presence of phytoconstituents like alkaloids, saponins, terpenoids, glycosides, flavonoids, sterols and steroids, tannins, phenolic compounds, carbohydrates¹⁰.

Table 2: Screening of Phytochemical constituents of *C. odorata*.

Phytochemical constituents	<i>C. odorata</i>					
	Petroleum Ether	Benzene	Chloroform	Ethyl Acetate	Ethanol	Water
Alkaloids	-	-	-	+	-	+
Saponins	-	+	-	-	+	+
Terpenoids	-	+	+	-	+	-
Glycosides	+	+	+	+	+	-
Flavonoids	+	-	+	-	+	-
Steroids and sterols	-	+	-	+	+	-
Tannins and phenols	-	-	+	+	+	+

“+” indicates presence of phytochemical constituents

“-” indicates the absence of phytochemical constituents

Agar Well Diffusion Method

The agar well diffusion method was used to determine the growth inhibition¹¹. The plant extracts were prepared at a concentration of 2.5, 5, 7.5, 10 µg/mL dissolved in Dimethyl Sulphoxide (DMSO) and it was tested. The DMSO act as control. The sterile Muller Hinton Agar was prepared and poured in sterile petri dishes and allowed to solidify. With the help of a sterile well cutter, 6mm diameter wells were punctured with uniform spacing for various concentrations for each extracts. The log phase culture broth was taken and swabbed over the plate using sterile cotton swab to obtain uniform lawn of culture. The wells were filled with 10 µl of the concentration of the plant extracts respectively. The plates were then incubated at 37°C for 24h for bacteria and at room temperature for yeast.

Susceptibility testing with Standard Antibiotics

The antimicrobial activity of standard antimicrobial agents like Bacitracin, Ampicillin, Nystatin, Nalidixic Acid, Erythromycin, Penicillin G, Kanamycin, Ciprofloxacin and Streptomycin were tested against pyogenic microorganisms. Their sensitivity pattern was compared using standard antibiogram chart.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the plant extracts were performed using broth dilution assay method¹². For assaying plant extracts, the starting concentration was kept at 8 mg/mL in the first tubes containing 1mL of broth. Tubes were vortexed to make the initial standard concentration. This was serially diluted to other tubes and finally 1mL was discarded from the last test tube hence making the dilution of 2, 1, 0.5, 0.25 µg/mL respectively. To all these test tubes, 0.1 mL of the log phase cultures of target microorganisms were added separately and incubated at 37°C for 24-48h. The tubes were examined for visible turbidity after incubation and plated on their respective media incubated at 37°C for 24h for bacteria and room temperature for yeast.

RESULTS

Organoleptic evaluation of *Chromolaena odorata*

The evaluation of the pharmacognostic character of the medicinal plant *C. odorata* was performed to test their sensory parameters (Table 1).

Screening for Phytochemical constituents

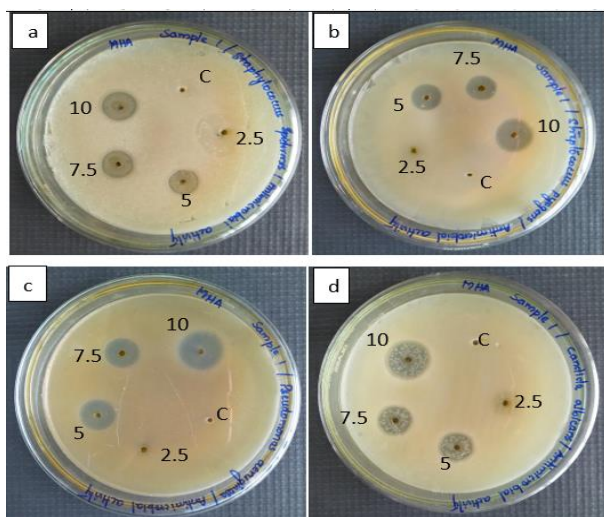


Figure 1: Antimicrobial activity of Petroleum ether extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 -10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL,2.-2.5 mg/mL.

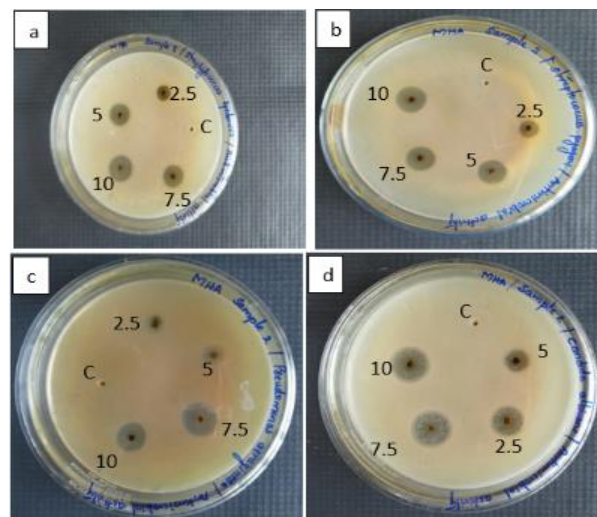


Figure 2: Antimicrobial activity of Benzene extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 -10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL,2.-2.5 mg/mL.

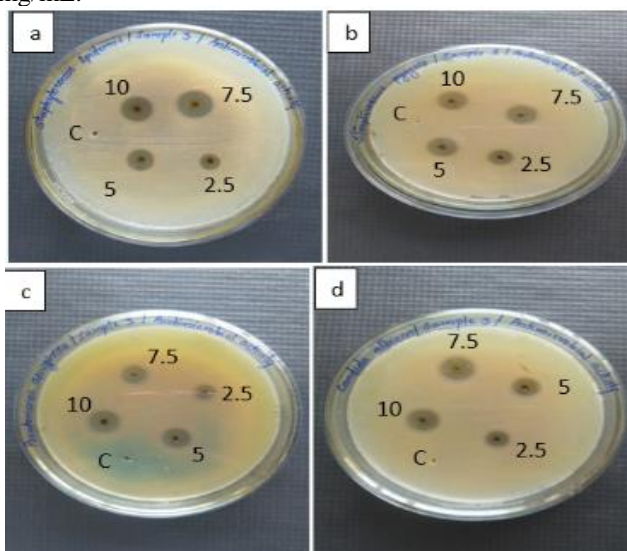


Figure 3: Antimicrobial activity of Chloroform extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 -10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL,2.-2.5 mg/mL.

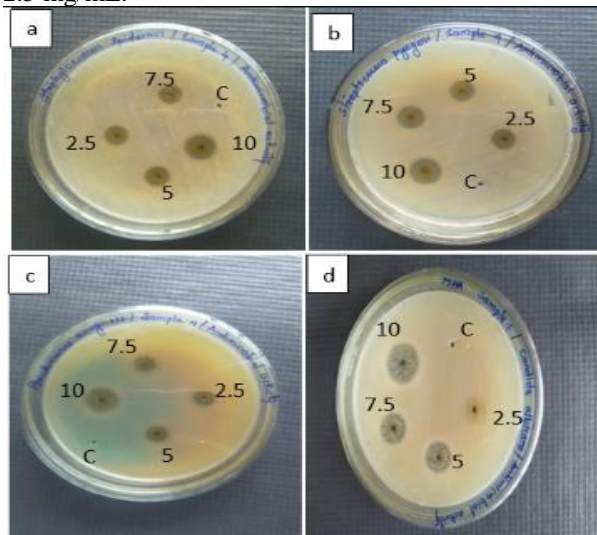


Figure 4: Antimicrobial activity of Ethyl Acetate extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 -10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL,2.-2.5 mg/mL.

The preliminary phytochemical screening of *C. odorata* extracts shows the presence of alkaloids, phenolic compounds, tannins, saponins, flavanoids and terpenoids compounds (Table 2).

Antimicrobial Activity and the Comparative Study Using Standard Antibiotics

The antimicrobial activity of *C.odorata* extracts were assayed by Agar well diffusion method. The extracts of the plant *C. odorata* showed higher activity against the selected pyogens. While comparing all the extracts, the ethanolic extracts of *C. odorata* had higher activity against the pyogenic pathogens, than the other extract.

Ethanol and benzene extracts of *C. odorata* had higher activity against *S. epidermidis* and *C. albicans* (Table 3). Ethanol and ethyl acetate extracts of *C. odorata* had higher activity against *S. pyogenes* and *P. aeruginosa* (Table 3). Ethyl acetate, chloroform and water extracts showed moderate activity against *S. epidermidis* and *C. albicans* (Table 3). Benzene, water and chloroform extracts showed moderate activity against *P. aeruginosa* and *S. pyogenes* (Table 3). Petroleum ether extracts showed less activity against the entire selected pyogenic pathogen (Table 3). The zone of inhibition of the plant extracts were compared with the commercial antibiotics and revealed that the plant

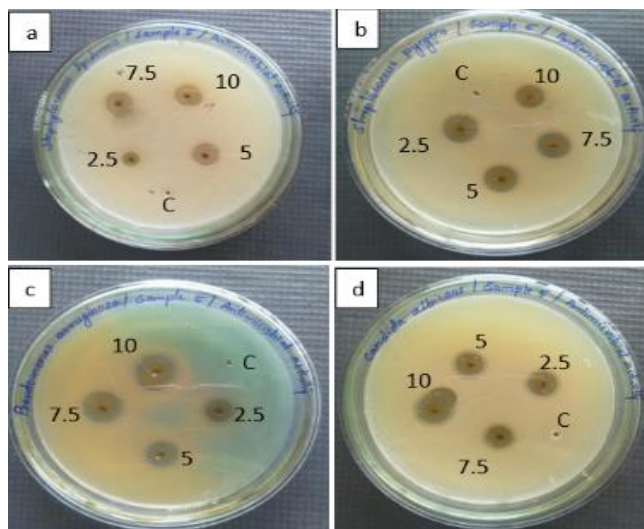


Figure 5: Antimicrobial activity of Ethanol extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 - 10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL, 2.5-2.5 mg/mL.

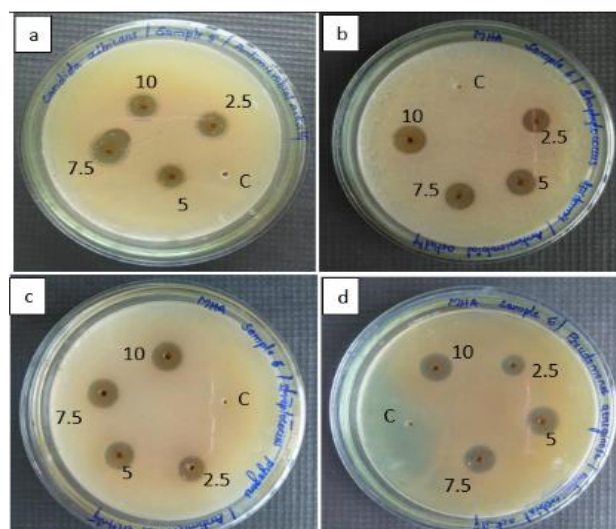


Figure 6: Antimicrobial activity of Water extract of *C.odorata* against Pyogenic Pathogens. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. C – Control, 10 - 10mg/mL, 7.5-7.5 mg/mL, 5-5 mg/mL, 2.5-2.5 mg/mL.

Table 3: Antimicrobial activity of *Chromolaena odorata* against pyogenic pathogen.

Sample	Organism	Zone of inhibition (diameter in mm)			
		10mg/mL	7.5mg /mL	5mg /mL	2.5mg /mL
Petroleum ether	<i>S. epidermidis</i>	12	11	8	No zone
	<i>S. pyogenes</i>	17	13	12	No zone
	<i>P. aeruginosa</i>	19	13	12	No zone
	<i>C. albicans</i>	16	13	12	No zone
Benzene	<i>S. epidermidis</i>	14	11	9	8
	<i>S. pyogenes</i>	13	11	10	8
	<i>P. aeruginosa</i>	14	12	7	5
	<i>C. albicans</i>	14	13	11	8
Chloroform	<i>S. epidermidis</i>	13	11	10	6
	<i>S. pyogenes</i>	12	11	10	8
	<i>P. aeruginosa</i>	13	12	10	8
	<i>C. albicans</i>	13	11	9	6
Ethyl acetate	<i>S. epidermidis</i>	12	10	9	7
	<i>S. pyogenes</i>	13	11	10	10
	<i>P. aeruginosa</i>	17	13	11	11
	<i>C. albicans</i>	12	10	9	7
Ethanol	<i>S. epidermidis</i>	13	12	11	9
	<i>S. pyogenes</i>	13	12	12	10
	<i>P. aeruginosa</i>	16	14	11	10
	<i>C. albicans</i>	13	11	10	9
Water	<i>S. epidermidis</i>	11	10	9	6
	<i>S. pyogenes</i>	10	9	8	8
	<i>P. aeruginosa</i>	13	12	11	9
	<i>C. albicans</i>	10	9	8	8

components had high inhibitory property than commercial antibiotics (Table 4) (Fig: 1, 2, 3, 4, 5, 6 & 7).

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration of the extract of *C.odorata* was done by plating method. After incubation the samples from the tubes were inoculated in the respective selective media and observed for visible growth.

There was no growth in the dilution of 2mg /mL. It indicated that the plant had effective phytochemical constituents against the pyogenic pathogens which were highly active even in low concentration. These results showed that the plant had high ethanobotanical value and can be used as phytomedicine (Table 5).

Table 4: Antimicrobial activity of Standard Antibiotic Disc on Pyogenic pathogen.

Antibiotic Disc	Zone of inhibition (diameter in mm)			
	<i>S.epidermidis</i>	<i>S.pyogenes</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Ampicillin (AMP ²⁵)	12 (Resistant)	11 (Resistant)	18 (Resistant)	10 (Resistant)
Bacitracin (B ¹⁰)	-	-	-	6 (Resistant)
Ciprofloxacin (CIP ⁵)	24 (Sensitive)	14 (Resistant)	23 (Sensitive)	19 (Intermediate)
Erythromycin (E ¹⁵)	-	13 (Resistant)	-	14 (Intermediate)
Kanamycin (K ³⁰)	22 (Sensitive)	16 (Intermediate)	-	14 (Intermediate)
Nalidixic acid (Na ³⁰)	9 (Resistant)	-	-	6 (Resistant)
Nystatin (Ns ⁵⁰)	-	-	-	7 (Resistant)
Penicillin G (P ¹⁰)	-	-	-	-
Streptomycin (S ²⁵)	19 (Sensitive)	13 (Resistant)	16 (Sensitive)	17 (Sensitive)

Table 5: Minimum Inhibitory Concentration of *C.odorata*.

Sample	Organism	Dilution (plating method)			
		T1 (2mg/mL)	T2 (1mg/mL)	T3 (0.5mg/mL)	T4 (0.25mg/mL)
Petroleum ether	<i>S. epidermidis</i>	--	--	+	+
	<i>S. pyogenes</i>	--	--	+	+
	<i>P. aeruginosa</i>	--	--	+	+
	<i>C. albicans</i>	--	--	+	+
Benzene	<i>S. epidermidis</i>	--	--	--	+
	<i>S. pyogenes</i>	--	--	--	+
	<i>P. aeruginosa</i>	--	--	+	+
	<i>C. albicans</i>	--	--	--	+
Chloroform	<i>S. epidermidis</i>	--	--	+	+
	<i>S. pyogenes</i>	--	--	--	+
	<i>P. aeruginosa</i>	--	--	--	+
	<i>C. albicans</i>	--	--	+	+
Ethyl acetate	<i>S. epidermidis</i>	--	--	--	+
	<i>S. pyogenes</i>	--	--	--	+
	<i>P. aeruginosa</i>	--	--	--	+
	<i>C. albicans</i>	--	--	--	+
Ethanol	<i>S. epidermidis</i>	--	--	--	+
	<i>S. pyogenes</i>	--	--	--	+
	<i>P. aeruginosa</i>	--	--	--	+
	<i>C. albicans</i>	--	--	--	+
Water	<i>S. epidermidis</i>	--	--	+	+
	<i>S. pyogenes</i>	--	--	--	+
	<i>P. aeruginosa</i>	--	--	+	+
	<i>C. albicans</i>	--	--	+	+

-- indicates No Growth on the plates, "+" indicates Growth on the plates

DISCUSSION

The present study was carried out to assess the antimicrobial activity of *C.odorata* against the pyogenic pathogen. The extracts of the leaves of *C.odorata* were prepared by successive solvent extraction method. The medicinal value of a plant can be determined on the basis of bioactive compounds. Phytochemical screening of *C.odorata* reveals that the presence of tannins, phenolic compounds, flavonoids, terpenoids, carbohydrate, glycosides, amino acids and proteins. The presence of these phytochemicals plays a vital role in the antimicrobial property of the plant. Alkaloids are major plant components responsible for antimicrobial activity. It intercalates with DNA and interferes with the cell division¹³. Flavonoids are a major group of plant phenolic compounds that act as antioxidant. It forms a complex with extracellular soluble proteins, bacterial cell wall and also disrupts the cell membrane¹⁴. Terpenoids are a subclass of

prenylipids and are naturally occurring organic chemicals. Its real mechanism is not fully understood but it damages the cell membrane^{15,16}. Tannins are polymeric phenolic substances which precipitate microbial protein and also inactivate microbial adhesion, enzymes, and cell envelope transport proteins. Moreover the presence of bioactive compounds and the supporting studies accompanied with them depicts that the *C.odorata* is highly active against pyogenic pathogens. It was reported that the plant extract have significant antimicrobial activity against Pyogenic microorganism due to the presence of various phytochemical constituents such as alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins¹⁷. This shows that the phytochemical constituents can be used to treat Pyogenic infection. The pH of the plant extract also plays a vital role in antimicrobial activity. Lower pH of the extract reduces the microbial load that is the extracts with acidic pH have more potency to kill microbes^{18,19}. The

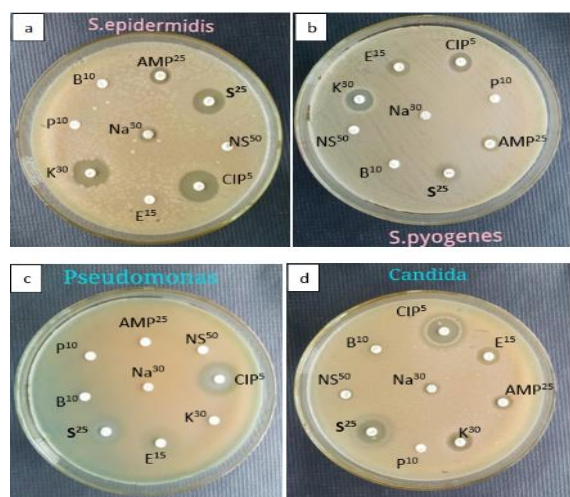


Figure 7: Antibiotic Susceptibility Testing against Pyogenic Pathogens using commercial antibiotics. (a) *Staphylococcus epidermidis*, (b) *Streptococcus pyogenes*, (c) *Pseudomonas aeruginosa* and (d) *Candida albicans*. CIP⁵- Ciprofloxacin (5mcg), B¹⁰- Bacitracin (10mcg), NS³⁰- Nystatin (30mcg), S²⁵- Streptomycin (25mcg), P¹⁰- Penicillin G (10mcg), K³⁰- Kanamycin (30mcg), AMP²⁵- Ampicillin (25mcg), E¹⁵- Erythromycin (15mcg), Na³⁰- Nalidixic acid (30mcg).

potency of the extract is also based on the method of extraction and concentration of plant extract²⁰. According to this information, it was clearly found that *C.odorata* has antimicrobial activity against the pyogenic pathogens. Among the extracts selected for the study, the ethanolic extract of *C.odorata* shows highest antimicrobial activity against the target microbial flora, when compared with other extracts. The ethanolic extract of *C.odorata* was found to be highly active against *Staphylococcus epidermidis*, *Streptococcus pyogenes*. *C. odorata* was highly active against *S. aureus* and *S. epidermidis*²¹. Ethanolic extract of the leaves of *C. odorata* have higher inhibitory activity against *S. aureus* when compared with standard antibiotics²². Combined ethanolic extracts of *J. curcus* and *C. odorata* have more potency with respect to antifungal activity. The combined extract of these plants reduce the growth of fungal pathogen significantly²³. It was compared with standard antimicrobial agents Ciprofloxacin (5mcg), Bacitracin (10mcg), Nystatin (30mcg), Streptomycin (25mcg), Penicillin G (10mcg), Kanamycin (30mcg), Ampicillin (25mcg), Erythromycin (15mcg) and Nalidixic acid (30mcg). MIC was taken as the lowest extract concentration that prevented the bacterial growth after 24h of incubation. After incubation the samples from the tubes were inoculated in respective selective media and observe for visible growth. There was no growth in the dilution of 2mg /mL. It indicates that the plant has effective phytochemical constituents against the pyogenic pathogens which were highly active even in low concentration. The presence of bioactive compounds and supporting studies reveals that antimicrobial activity of *C. odorata* which can be used to discover bioactive natural products.

CONCLUSION

The antimicrobial assessment of *C.odorata* against the selected pyogens reveals that the phytoconstituents has high antimicrobial property which was compared and confirmed with commercial antibiogram. Since the drug resistance nature of the pyogens increases day by day this herbal remedies will serve as a alternative medicine without side effects. These extracts were used as medicine for pyogenic infection instead of using synthetic antibiotics and drugs. These plants can be used to discover bioactive natural products that serve as lead for the development of new phytopharmaceuticals.

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