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ANTIMICROBIAL ACTIVITY OF *CHROMOLAENA ODORATA* AGAINST WOUND INFECTION

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Abstract: This plant extract that was well-trying to be effectively used as a natural different supply of stop and cure the wound accustomed discover different bioactive natural product that will function lead for the event of latest pharmaceutical drug. Hence, this plant may function an alternate medication while not facet effects and additionally they might more be vulnerable bacterium were *Escherichia coli*. The recent ethanolic extract solely suppressed the expansion of staph aureus. dry ethanolic extract suppressed the expansion of staph aureus, bacteria genus spp, *E. coli*, *Klebsiella*. Spp. the foremost properties by agar well diffusion technique mistreatment Mueller-Hinton Agar (AHM) on human infective bacterium. The dry and recent ethanolic extracts of the leaves of *Chromolaena odorata* were studied for in vitro antimicrobial

KEYWORDS: *C. odorata*, Antimicrobial activity, wound infection, MIC

I. INTRODUCTION

Pyogenic infection is associated in nursing infection characterized by severe native inflammation, typically with pus formation, generally caused by the pyogenic organism. Humans area unit 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, significantly those involving the skin and mucosal membrane surfaces might cause serious complications. The foremost common bacterium that as a result of wound infections are *Staphylococcus aureus*,

Staphylococcus epidermidis, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*.

S. epidermidis is an endemic organism, consisting of non-motile Gram-positive cocci, arranged in grape like clusters. The ability to makes biofilms on plastic devices may be a major virulence issue 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 animals. Traditional medicative plants area unit for the treatment of wound infection with unsafe effects in effects in body. Medicative 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 hazard division have antimicrobial activity against some selective organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli*. *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 strains. The main aim of the study is to determine the efficacy of the plant extract *Chromolaena odorata* against the Pathology infective agent.

II. OBJECTIVE

- To isolate and characterise of wound infected samples.



- To test the antibacterial activity of *Chromolaena odorata* against the wound infected pathogen.
- To find out MIC of isolated pathogens.

III. MATERIALS AND METHODS

Collection of samples

Completely different wound swab samples were collected for the isolation of pathology bacteria that infect the wounds.

Isolation of organisms

The collected samples were inoculated into blood agar and MacConkey's Agar. The inoculated media were incubated overnight at 37°C for 48 hours.

Identification of organism

The isolated organisms were known by following techniques (Berge's manual) of microscopic observation and cultural characteristics, biochemical characteristics.

Microscopic observation

Gram's staining

Gram's staining was performed for the colonies obtained.

Cultural characterization

The observed microorganisms were inoculated in to respective media and incubated at 37°C.

Biochemical characterization

Biochemical characteristics were performed.

Collection of Leaf

Collection and Storage of Plant Materials

The leaves of *C. odorata* were collected from nearby areas. It was washed in running water to induce eliminate dirt particles and subjected to shade drying for concerning one week. Dried leaves were powdered with an electric mill. The sample were stored in an airtight container and tested for certain biologically active compounds.

Organoleptic analysis of C. odorata

The plant parts were organoleptically evaluated and examined for numerous sensory parameters like colour, appearance of the plant parts principally size and shape, external texture, fracture (granular, splintery, smooth) and external markings (furrow, wrinkles, ridges, annular, out growth) of the plant components. The fragrance, test for odour (aromatic, balsamic,

camphorates, spicy, pleasant, irritating) and taste (sweet, bitter, sour, astringent, pungent, acidic, alkaline) were evaluated.

Successive Solvent Extraction of plant materials

The phytochemical constituents were extracted using successive solvent extraction method based on polarity. About 20 grams of grained leaves of *C. odorata* was separately mixed with 100mL of solvent (Petroleum Benzene, Chloroform, Ethanol, Water) and subjected to occasional shaking for 24h. The plant extracts were filtered using Whatman 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 completely to eliminate the trace of solvent used.

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 resin compounds, carbohydrates

IV. METHODOLOGY FOR THE LCMS ANALYSIS

Sample: Chromolaena

SPECIFICATIONS:

Lc column : reverse phase c-18

Pump : spd 10 avp

Mobile phase : water& methanol (50:50)

Ionization mode : electronic spray ionization/APCI

Mode : both positive and negative

Injection volume : 10microlitre

Flow rate : 2 ml/min

Column temperature : 250c

Column : Phenomenex rp 18

Column dimension : 25 cm x2.5 mm

Lc detection : 254 nm

M/z range : 50-800 for neg and 50-950 for pos

Software : class v p integrated.

Library : Met win 2.0

V. ANTIBACTERIAL ACTIVITY OF *C. ODERATA*

Agar Well Diffusion Method

The agar well diffusion method was used to determine the expansion inhibition. The plant extracts were prepared at a concentration of two, 5, 5, 7.5, 10 µg/mL. The sterile Muller Hinton Agar was prepared and poured in sterile petri dishes and allowed to solidify. With the assistance of a sterile well cutter, 6mm diameter wells were punctured with uniform spacing for various

concentrations for every extract. The log phase culture broth was taken and swabbed over the plate using sterile cotton swab to get uniform lawn of culture. The wells were crammed 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.

Table-1 Morphological, Cultural, Physiological and Biochemical Characteristics of the Isolated Strains

CHARACTERISTICS	Sample 1		Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
	MacConkey	Blood Agar							
Colony morphology	No Growth	No Growth	No Growth	Golden yellow coloured colonies		Circular, Mucoid, Greyish white	Light golden yellow, surrounded by clear zone (beta hemolysis).	Light golden yellow, surrounded by clear zone (beta hemolysis).	
	No Growth	No Growth	No Growth	Circular, Pink, Convex		Circular, mucoid, Pink Red, Convex	Circular, Pink, Convex	Circular, Pink, Convex	
Gram's staining	NA	NA	NA	G+	G-	-	G+	G+	-
Indole test	NA	NA	NA	-	+	-	-	-	-
MR	NA	NA	NA	+	+	-	+	+	-
VP	NA	NA	NA	+	-	+	+	+	+
Citrate	NA	NA	NA	+	-	+	+	+	+
Oxidase	NA	NA	NA	-	-	-	-	-	+
Catalase	NA	NA	NA	+	+	+	+	+	+
TSI	NA	NA	NA	A+G-	Acid Butt, Acid Slant	Acid Butt, Acid slant	A+G-	A+G-	Alkaline Butt, Alkaline Slant
Motility	NA	NA	NA	-	A+G-	A+G-	A+G-	A+G-	+

Susceptibility testing with Standard Antibiotics

The antimicrobial activity of ordinary antimicrobial agents like Ampicillin, Bacitracin, Ciprofloxacin, Gentamicin and Levofloxacin were tested against wound infecting microorganisms. Their sensitivity pattern was compared using standard antibiogram chart.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of all the Supernatant decided for every of the test organisms in triplicates at varying concentration of 1%, 3%, and 5%. To get this varying concentration added 10ml of peptone water. A tube containing peptone water only was seeded with the test organism to function control. All the tubes were then incubated at 37°C for twenty-four hours then examined for growth by observing turbidity.

VI. PREPERATION OF DISC

Disc cutting to the gauze in circular shape size (6mm). Then disc is dipped in already prepared leaf extraction (petroleum, ethanol. etc.). After 5 minutes disc allow to air dry.

VII. DISC DIFFUSION METHOD

18 hours broth cultures of every test organism were inoculated into Muller Hinton agar using sterile cotton swab. The three concentrations of discs dipped in each test plate using sterile forceps. Then the plates were incubated at 37°C for twenty-four hours. After the incubation period, the diameter of inhibition zones of every disc was measured.

Isolation and identification of organism.

From the collected samples, the subsequent organisms were isolated.

1. *Staphylococcus aureus*
2. *Pseudomonas spp*
3. *E. Coli*
4. *Klebsiella. Spp.*

Antimicrobial activity of *C. Odorata* against wound infecting pathogens

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 wound infecting pyrogens. While comparing all the extracts, Ethanol and Chloroform extracts of *C. odorata* had higher activity against the wound infecting pathogens. Water and Petroleum ether extracts showed less activity against the wound infecting pathogen. The zone of inhibition of the plant extracts were compared with the commercial antibiotics and revealed that the plant components had high inhibitory properties

Antimicrobial activity of *Chromolaena odorata* against wound infecting pathogen.

Table -2: Petroleum benzene Extract of *C. Odorata*

ORGANISM S	ZONE OF INHIBITION			
	10 mg/m L	7.5 mg/m l	5 mg/m l	2.5 mg/m l
<i>S. aureus</i>	15	13	11	-
<i>E. coli</i>	13	11	8	8
<i>Klebsiella spp</i>	12	11	8	8
<i>S. aureus</i>	14	13	11	11
<i>S. aureus</i>	15	14	13	11
<i>Pseudomonas spp</i>	10	8	-	-

Table-3 Ethanol Extract of *C. Odorata*

ORGANISM S	ZONE OF INHIBITION			
	10 mg/m L	7.5 mg/m l	5 mg/m l	2.5 mg/m l
<i>S. aureus</i>	20	14	11	8
<i>E. coli</i>	11	8	-	-
<i>Klebsiella spp</i>	12	8	6	-
<i>S. aureus</i>	21	16	14	8
<i>S. aureus</i>	12	15	14	11
<i>Pseudomonas spp</i>	13	10	-	-

Table-4: Distilled Water Extract of *C. Odorata*

ORGANISMS	ZONE OF INHIBITION			
	10 mg/mL	7.5 mg/ml	5 mg/ml	2.5 mg/ml
<i>S. aureus</i>	17	12	10	-
<i>E. coli</i>	16	12	-	-
<i>Klebsiella spp</i>	14	9	-	-
<i>S. aureus</i>	15	12	10	-
<i>S. aureus</i>	16	12	10	-

<i>Pseudomonas spp</i>	14	12	10	-
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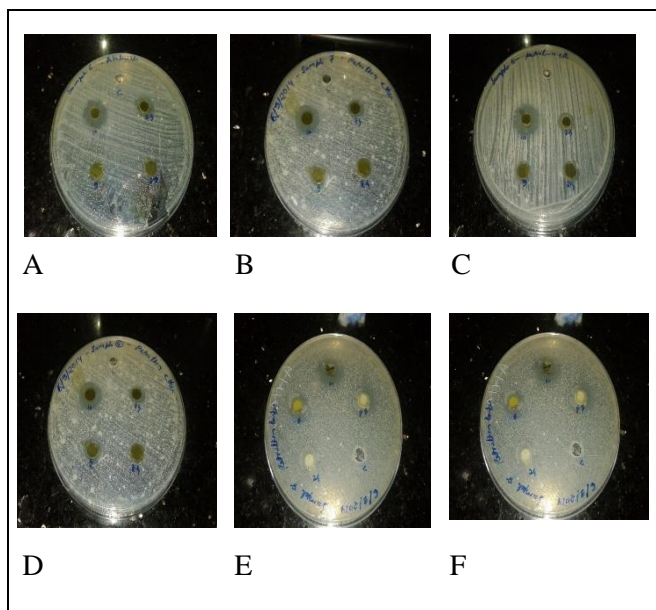


Fig. 1: Antimicrobial Activity of Petroleum Benzene Extract of *Chromolaena Odorata* Against Wound Infecting Pathogens (a) *Staphylococcus aureus*, (b) *E. coli*, (c) *Klebsiella*, (d) *Staphylococcus aureus*, (e) *Staphylococcus aureus*, (f) *Pseudomonas spp*

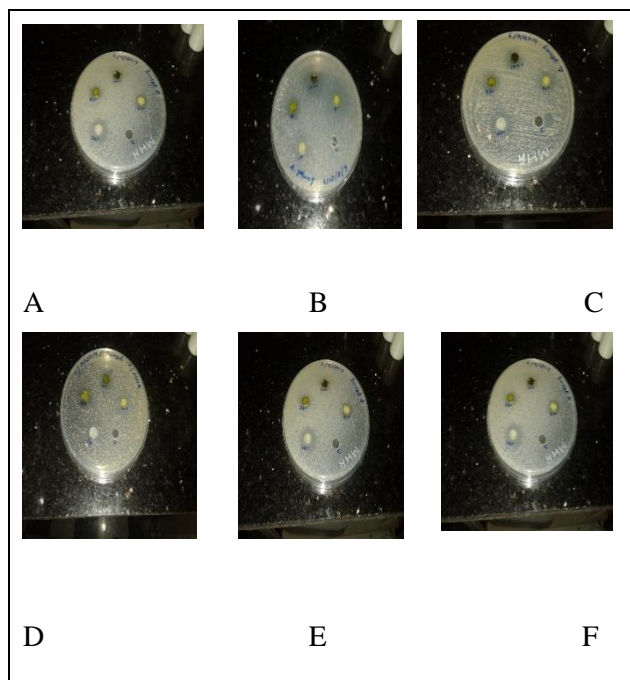
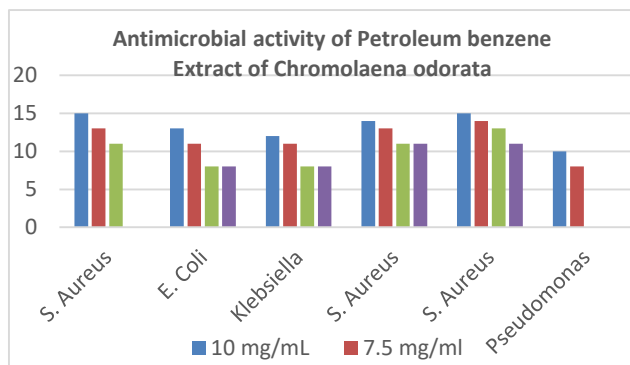
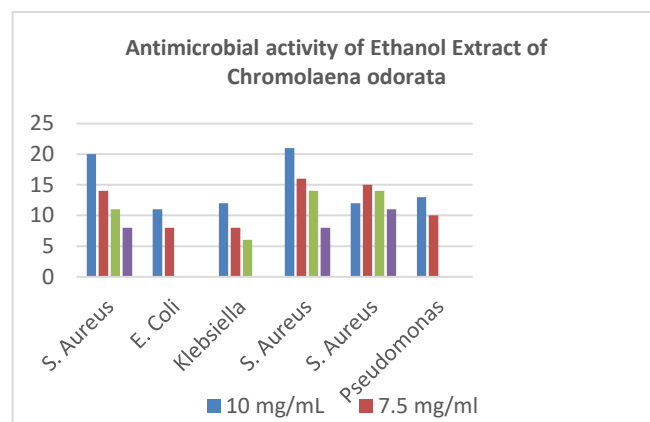


Fig. 2: Antimicrobial Activity of Chloroform Extract of *Chromolaena Odorata* Against Wound Infecting Pathogens (a) *Staphylococcus aureus*, (b) *E. coli*, (c) *Klebsiella*, (d) *Staphylococcus aureus*, (e) *Staphylococcus aureus*, (f) *Pseudomonas spp*.



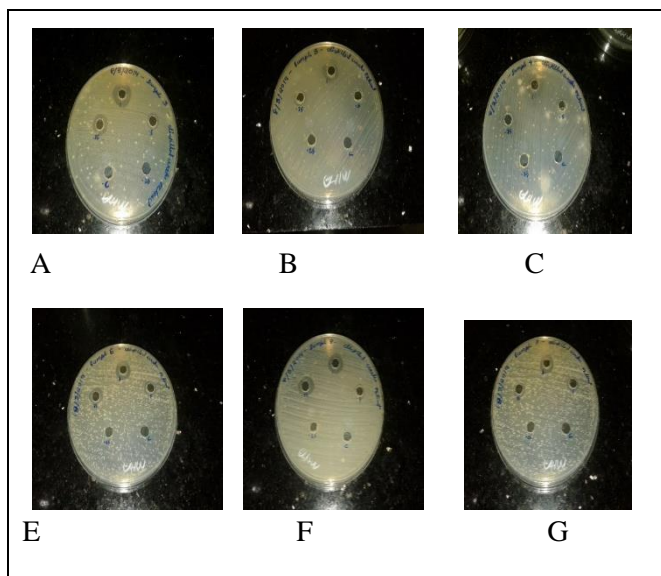
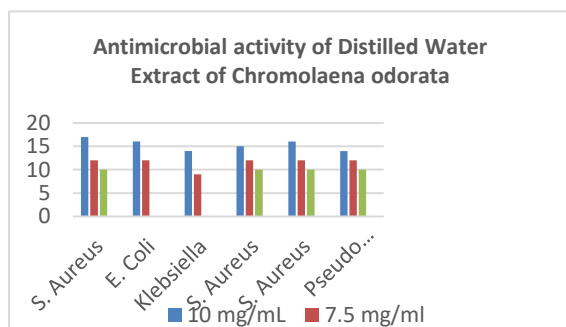


Fig. 3: Antimicrobial Activity of distilled water Extract of *Chromolaena Odorata* Against Wound Infecting Pathogens (a) *Staphylococcus aureus*, (b) *E. coli*, (c) *Klebsiella*, (d) *Staphylococcus aureus*, (e) *Staphylococcus aureus*, (f) *Pseudomonas spp*



VIII. RESULT AND DISCUSSION

The antimicrobial assessment of *C. Odorata* against the wound infecting pathogens reveals that the phytoconstituents has high antimicrobial property which was compared and confirmed with commercial antibiogram. Since the drug resistance nature of the pyrogens increases day by day this herbal remedy will function an alternate medicine without side effects. These extracts were used as medicine for pyogenic infection rather than using synthetic antibiotics and medicines. These plants are often went to discover bioactive natural products that function lead for the event of latest phytopharmaceuticals.

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