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From plant to potent: Evaluating the antimicrobial and antioxidant capacity of leaves extract from *Coccinia grandis* (L)

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Abstract
Coccinia grandis (L) is the most valuable plant and is used to treat a variety of illnesses. Various therapeutic disorders are treated with bioactive compounds originating from plants. Investigating antibacterial and antioxidant activity using disc diffusion and DPPH scavenging, respectively, is the goal of the current work. All four bacterial species were active against the crude extract. Gram-negative bacteria include *Proteus vulgaris* and *Escherichia coli*, while gram-positive bacteria include *Staphylococcus aureus* and *Bacillus subtilis*. The leaves extract has strong antibacterial activity against *S. aureus* and *E. coli*, as evidenced by the most notable inhibitory zones they showed. Conversely, *P. vulgaris* and *B. subtilis* showed mild inhibitory zones, indicating that the leaves have a moderate antibacterial action on these bacteria.The micro broth dilution method was used to find the aqueous extract's least inhibitory concentration (MIC). The extracts were serially diluted twice for MIC (200, 100, 50, 25, and 12.5 µg/ml). For 18 to 24 hours, the tubes were incubated for bacteria at 370C, and any growth that was evident was monitored.

Keywords: Bioactive compounds, DPPH, *Proteus vulgaris,Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* MIC

Introduction
Bacterial infections have been an issue for humanity since the beginning of time. Antimicrobial medicine research has been underway for more than 50 years [1]. Antibiotic-resistant bacterial infections and the adverse effects of antibiotic treatment for people who are allergic to them persist despite the discovery of numerous medications [2]. Health practices, methods, knowledge, and beliefs that use plant and mineral-based medicine to treat, diagnose, and prevent disease or preserve health are referred to as traditional plant medicine [3]. Numerous Nepalese ethno medicinal plants have been identified, and their uses have been documented [4]. These known plants have been utilized for general medicinal purposes, including antiviral, antifungal, and antibacterial properties [5]. However, there is currently a dearth of a comprehensive and scientific study on the antibacterial qualities of medicinal plants found in northern Maharashtra [6]. The main function of antimicrobial agents is to lessen the prevalence of infectious diseases worldwide [7]. However, antibiotics lose some of their potency as resistant organisms grow and proliferate. Once people realised that the effective life span of antibiotics was limited and that the overuse and abuse of traditional antibiotics was leading to germ resistance, the use of plant extracts for therapeutic purposes gained popularity [8]. The application of phytochemicals and crude extracts of plant components with established antibacterial qualities is crucial to therapeutic interventions [9]. Extraction is the process of utilising a selective solvent called menstruum and established techniques to separate the therapeutically active parts of plant tissues [10]. A complex variety of medicinal plant metabolites, including flavonoids, tannins, lignans, alkaloids, glycosides, and terpenoids, are present in the goods [11]. To become a modern medication, an extract can be further processed using different fractionation procedures to separate specific chemical entities including codeine, hyoscine, hyoscyamine, vincristine, and vinblastine [12]. In this study, an effort has been made to determine the lowest inhibitory concentration of plant extracts and assess the antibacterial activity of extracts of *Coccinia grandis L* leaves against human hazardous bacteria.

The purpose of this is to promote the use of medicinal herbs that are readily available in the area rather than antibiotics, which can have a number of negative effects.

Using axillary tendrils, *C. grandis* is a sub-woody perennial climber or creeper that can grow up to 20 to 25 meters in length. Stems with white spots and many branches can reach a length of 5 meters. Tendrils that are simple or bifid are shorter than leaves [13]. Five triangular lobes adorn the simple alternate leaf blades (3 to11 cm × 3 to 13 cm), while the glabrous petioles measure 1.5 to 2 cm in length. The corolla, which is 2 to 4 cm long and white with a pale yellow center, has single blooms [14]. The ellipsoid fruits range in length from 3 to 6 cm. ripe fruits are red, whereas unripe fruits are greenish with pale streaks. *C. grandis* has been used traditionally as a herbal remedy for several conditions, including diabetes, high blood pressure, jaundice, and building muscle [15]. The most widespread of them is the usage of this plant to treat diabetes in several traditional medical systems, which prompted researchers to look into the validity of this use [16]. Studies have demonstrated that *C. grandis* is an effective therapeutic agent for diabetes and other disorders linked to diabetes, including inflammation, oxidative stress, dyslipidaemia, and obesity [17]. The existence of alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, and glycosides in *C. grandis* was demonstrated by phytochemical analysis. The plant's many pharmacological properties, including its hypoglycemic, anticancer, antinociceptive, antipyretic, anthelmintic, analgesic, spasmolytic, wound-healing, antiulcerogenic, anti-convulsant, hepatoprotective, and immunomodulatory effects, are caused by these bioactive chemicals. A thorough assessment of the body of research on the pharmacological actions, phytochemical analysis, and safety profile of the traditional medicinal herb *C. grandis* is given in this work. This information will be useful to researchers as they investigate the plant's pharmacological potential, look into specific pathways, and create safe and efficient plant-based medications [18].

Fig 1: Plant parts of *Coccinia grandis (L)*

Classification

- **Scientific Classification**
- **Kingdom:** Plantae
- **Clade:** Tracheophytes
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Rosids
- **Order:** Cucurbitales
- **Family:** Cucurbitaceae
- **Genus:** Coccinia
- **Species:** *C. grandis*
- **Binomial Name:** *Coccnia grandis (L)*

Material and Methods

Collection of plant samples and bacterial strains

Various locations in Nashik, Dhule, Jalgaon, and Nandurbar district of Maharashtra, India, provided plant samples for the study, which was conducted in the chemical lab of Rani Laxmibai Mahavidyalaya, Pafrola, Jalgaon (MH), India. To test the antibacterial activity of plant extracts, NCIM strains of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Candida albicans, and Aspergillus niger—all sourced from the National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 India were employed [19].

Extraction of antimicrobial compounds:

The methanolic extracts of medicinal herbs were made by separately dissolving 20 grammes of dry powder in 100 millilitres of methanol. The mixture was then stored in a separating funnel for seven days, shaking constantly every twenty-four hours. After seven days, the extract was filtered, allowing the remaining ethanol to evaporate at the evaporator while the sediment sank at the bottom. The leftover filtrate, known as extract, which included the chemical components of each medicinal plant, was dried at 400 degrees Celsius in a hot air furnace and refrigerated for later use. After being sterilised, Whatman's No. 3-filter paper was punched into a 2mm disc. The extract concentrations, which were made with DMSO and methanol in a 10:90 ratio, were applied to each disc in the following concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml [20].

Disc diffusion method

100µl of a new culture of human pathogens was swabbed onto each sterile petri plate after 20ml of sterilised Muller Hinton Agar had been added and the cation had solidified. Using sterile forceps, the 6 mm discs were held over the agar plates and incubated for 24 hours at 370 C. Following incubation, measurements were made of the inhibitory zone diameter [21].

Standardization of bacterial suspension

Amphotericin-B standard was used as a reference to adjust the turbidity of bacterial suspensions. The bacterial suspensions were standardized following the CLSI guidelines for aerobic bacteria [22].

Cultures used

Microorganism	Strain Name	Strain reference
Gram positive bacteria	*Staphylococcus aureus*	NCIM 2079
	Bacillus subtilis	NCIM 2063
Gram negative bacteria	*Escherichia coli*	NCIM 2109
	Proteus vulgaris	NCIM 2172
Fungi	*Candida albicans*	NCIM 3471
	Aspergillus niger	NCIM 545
NCIM: National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 [India]		

Concentration of compounds

Stock solution [1000 microgram per ml] of each compound was prepared in Distilled water. Assay carried out by taking concentration 100 microrgram per disk. Hi-media antibiotics disk Chloramphenicol (10 microgram/disk), moistened with water are used as standard.

Media used

- Microbiological media used for bacteria is Nutrient agar (Hi-media) Composition (gL-1): Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2).
- Microbiological media for fungi and yeast is Potato dextrose agar (all ingredients of Hi media) Composition (gL-1): Potatoes infusion 200; Dextrose 20; Agar 15; Final pH (at 25^0C) 5.6±0.2

Determination of MIC

The micro broth dilution method was used to find the methanol extract's minimum inhibitory concentration (MIC) (Andrews JM 2001). The extracts were serially diluted twice for MIC (200, 100, 50, 25, and 12.5 µg/ml). For 18 to 24 hours, the tubes were incubated for bacteria at 370C, and any growth that was evident was monitored. Bacterial suspensions served as the positive control, while broth extracts served as the negative control. The minimum inhibitory concentration (MIC) was defined as the extract concentration at which no discernible growth occurred in comparison to control tubes that contained only the extracts [23].

Results

Table 1: Antibacterial activity of the leaves extract form *Coccinia grandis (L)*

Sr. No.	Name of the Organism	Chloramphenicol (standard)	Inhibition Zone (mm)
1	*E. Coli*	25.97	11.99
2	*P. Vulgaris*	22.36	8.52
3	*S. Aureus*	31.35	15.31
4	*B. Subtilis*	22.87	8.97

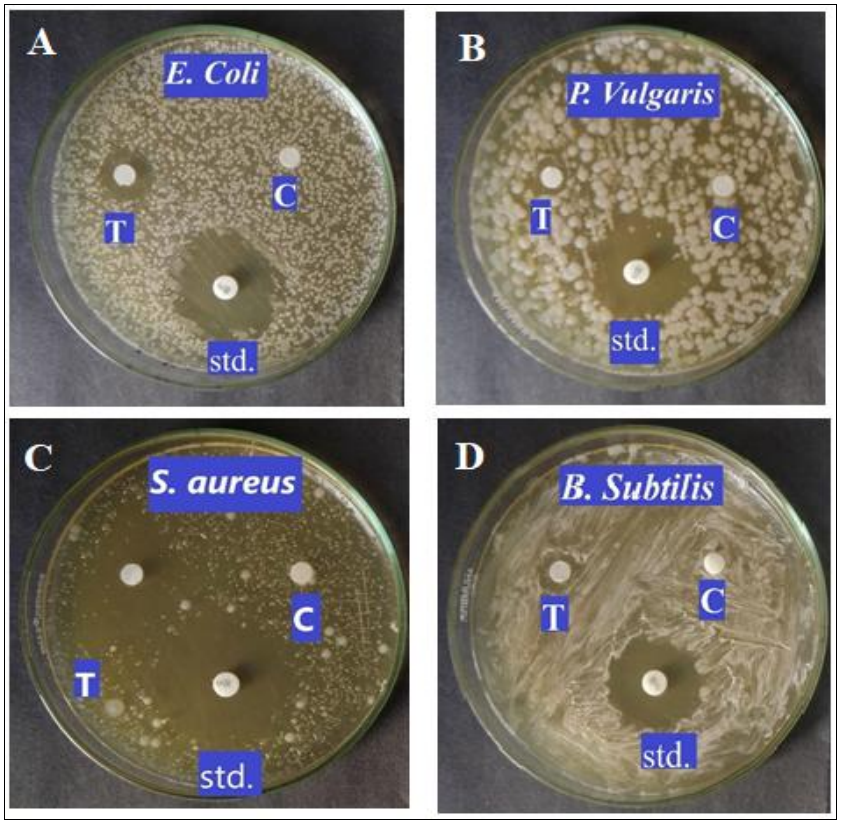

Fig 2: Antibacterial activity of leaf extract from *Coccinia grandis (L)* against human pathogens. T- leaf extract, C- DMSO control, std. – standard (Chloramphenicol)

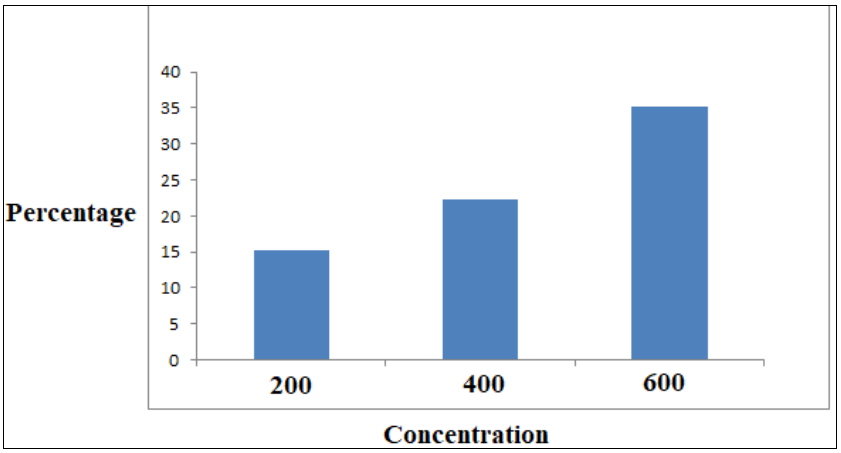

Fig 3: Antioxidant activity by DPPH Method

Discussion

Leaves extract was meticulously prepared in methanol for the assessment of antibacterial efficacy against four distinct bacterial strains. The crude extract exhibited activity against all four bacterial species. St*aphylococcus aureus* and *Bacillus subtilis* are categorized as gram-positive bacteria, whereas *Escherichia coli* and *Proteus vulgaris* are classified as gram-negative bacteria. Notably, S. aureus and E. coli demonstrated the most significant inhibition zones, indicating that the leaf extract possesses robust antibacterial activity against these pathogens. In contrast, B. subtilis and P. vulgaris exhibited moderate inhibition zones, suggesting that the crab tissue exerts a moderate antibacterial effect against these bacteria (Table 1). The leaf extract yielded the highest inhibition zones of 15.31 mm and 11.99 mm against S. aureus and E. coli, respectively, while showing moderate inhibition against B. subtilis and P. vulgaris. (Fig. 2).

Plants are abundant sources of bioactive compounds, with a significant proportion derived from marine biota. However, challenges persist in the determination of structures, synthesis, and bioactivities of these compounds. Marine organisms have garnered considerable attention in drug discovery due to their clinical potential. This field has become a focal point for research across various disciplines, including chemistry, biology, biochemistry, and biotechnology. Numerous studies have documented the antimicrobial properties exhibited by various marine crustaceans against both Gram-positive and Gram-negative bacteria.

Peptides isolated from several crab species have demonstrated antifungal activity against human pathogens, prompting extensive exploration for drug development. In this research, the antimicrobial efficacy of crude extracts from the leaves of *Coccinia grandis (L)*. was investigated against various human pathogens. This study employed both bacterial and fungal strains, encompassing a range of Gram-positive and Gram-negative bacteria. Specifically,*E. coli* and *P. vulgaris* represented Gram-negative bacteria, while *S. aureu*s and *B. subtilis* were categorized as Gram-positive. Among these bacterial strains, the crude tissue extract exhibited a notable inhibition zone of 15.31 mm against *S. aureus*, while the inhibition zone against *E. coli* measured 11.99 mm, indicating strong antimicrobial activity. Conversely, the Gram-positive bacterium *B. subtilis* displayed an inhibition zone of 8.97 mm, and the Gram-negative bacterium *P. vulgari*s showed a marginally lower inhibition zone of 8.52 mm in response to the extract, suggesting a comparatively weaker activity against these organisms.

DPPH radical scavenging activity in the leaves demonstrated a value of 15.17% when juxtaposed with the standard of 22.29% at a concentration of 200µg/ml. At 400µg/ml, the activity was recorded at 22.22%, in contrast to the standard of 27.96%. Furthermore, at 600µg/ml, a scavenging activity of 35.22% was observed, compared to the standard of 33.77%. This suggests a slight decline in radical scavenging absorption activity in the leaves relative to the standard absorption. Conversely, the stems exhibited a pronounced reduction in radical scavenging activity, as illustrated in the accompanying figure. The data reveal that at a concentration of 200µg/ml, the absorption was recorded at 0%, in stark contrast to the standard of 22.29%. For the 400µg/ml concentration, the absorption was a mere 3.03% compared to 27.96%, and at 600µg/ml, only 11.21% was noted against the standard radical scavenging activity of 33.77%.

Conclusion

From the present study, we deduce that *Coccinia grandis (L)* leaves exhibits substantial activity against the pathogen, signifying the presence of numerous bioactive compounds that warrant exploration for drug development. The selected microorganisms can be the focal point for investigating biosynthetic genes, and the volatile compounds affirm their potential as a viable source of antimicrobial agents. Further research is imperative to advance the development of naturally potent pharmaceuticals. The antioxidant study has elucidated the significance of traditional medicine in the treatment of various bacterial diseases, offering advantages over antibiotics by shortening the duration of treatment, enhancing patient compliance, and mitigating the risk of overdose, which may result in toxicity or other adverse effects.

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Conflict of Interest

Not available.

Financial Support

Not available.

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