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ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial activity of β-citronellol against Gram-positive bacteria and Gram-negative bacteria

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ABSTRACT

Bacterial infection and the HPV virus are thought to have a greater connection. This bacterial pathogen frequently causes infections that can be acquired both outside of the hospital and in the community. The development of multi-drug resistant strains like MRSA (Methicillin-Resistant Staphylococcus aureus) makes treatment difficult. The creation of new antibacterial agents is the primary tactic used to combat the issue of bacterial resistance. A terpene renowned for its ability to fight cancer is β-citronellol. The antibacterial activity of the chemical was the focus of the current investigation. The antibacterial activity of β-citronellol against Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa), including Ciprofloxacin strains, is described in the current work. Gram-positive as well as Gram-negative bacteria were susceptible to the substance's antibacterial effects. The study concludes the compound's antibacterial characteristics and recommends more research into the compound's possible bioactivities.

Keywords: Cervical cancer, Human papillomavirus, *Ficus racemosa*, β-citronellol, bacteria.

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INTRODUCTION

Squamous intraepithelial lesions (SILs) particularly cervical cancer risk have been linked to a variety of characteristics, including early age of first sexual activity, smoking, many sexual partners, and poor socioeconomic level. However, cervical cancer as well as its precursor lesions, SIL or cervical intraepithelial neoplasia (CIN), are caused by the human papillomavirus (HPV) [1][2]. Only 10% to 20% of infected females continue to have HPV infections after one or two years, and the majority of cases are asymptomatic [3][4]. The disorder known as bacterial vaginosis (BV) is curable and is characterized by disturbance of the environment of the vagina and the typical vaginal flora, which consists primarily of lactobacilli, being replaced by a variety of anaerobic organisms [5]. Most BV cases in women of childbearing age are thought to be associated with hormonal changes (changes in vaginal pH) and sexual conduct. Since BV is commonly identified in low-grade SILs, it has been hypothesized that BV may play a significant role in the formation of SIL.

There is evidence that an infectious agent is the cause of more than 15% of cancers globally [6]. The primary ways that infections result in cancer include (a) the transformation of cells by the introduction of oncogenes and/or the suppression of tumor suppressors (for example, oncogenic HPV types, the main cause of cervical cancer, linked with the tumor suppressor protein p53 and aim it towards toxicity; [7] (b) immunosuppression-induced carcinogenesis, such as in malignancies linked to HIV [8], and (c) chronic inflammation brought on by an infection can generate cytokines and nitric oxide that aid in the development of cancer [9][10] (e.g., The first bacteria to be definitively linked to human cancer, according to the IARC, is Helicobacter pylori [11] generates a long-lasting inflammatory reaction in the stomach mucosa, which is probably what causes cancer to grow [12].

Lactobacilli are known to predominate over other bacteria in a healthy vaginal microbiome. Changes in the balance of the vaginal microbiota can lead to poly-bacterial dysbiosis, which is a changed composition, and diseases like bacterial vaginosis, both of which have been linked to vaginal HPV infection [13][14]. The diversity of the cervicovaginal microbiota is higher in people with HPV infection, and HPV clearance has been linked to an increase in antigen-representing Langerhans cells [15].

Among the pathogens that are the source for diseases are caused by resistant bacteria are the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species). These infections are linked to lengthier hospital stays, higher hospital expenses, greater use of antimicrobial medications, and higher fatality rates.

A gram-positive bacterium called *Staphylococcus aureus* is responsible for a wide range of clinical illnesses. This bacterium frequently causes infections, both in situations where they are contracted in the community and hospitals. The development of multi-drug resistant strains like MRSA (Methicillin-Resistant *Staphylococcus aureus*) makes treatment difficult.

Pseudomonas aeruginosa is a gram-negative, aerobic, non-spore-producing rod that may infect both immunocompetent and immunocompromised hosts with a range of diseases. It is a very difficult organism to treat in modern medicine due to its propensity to infect immunocompromised hosts, high flexibility, and antibiotic resistance, with a wide spectrum of dynamic defenses.

In bacterial strains isolated from cervical cancer biopsies, the HPV-16 genome was found. It was also shown that the HPV-16 genes could be translated and transcribed in these bacterial strains, as well as that the HPV-16 gene product could produce virus particles in these bacteria. This is the first study to demonstrate the presence of HPV in bacteria extracted from human tissue samples. However, as it only verifies the presence of HPV-16 in the particular bacterial strains that were recovered, more research is necessary to understand this phenomenon [16].

The creation of novel antibacterial agents is the primary tactic used to combat the issue of bacterial resistance. The synthesis of novel compounds and modification of existing ones is promising concerning this technique and can increase the alternatives for new medications with a wider range of activity, lower toxicity, and/or reduced vulnerability to resistance mechanisms [17].

New antibacterial medicines for clinical use have been introduced as a consequence of this strategy, including retapamulin, a substance derived from pleuromutilin, and some of the traditional modifications of penicillins, the aminopenicillins [18][19].

In the essential oil of plants in the genus, β-citronellol (Figure 1) is a monoterpene alcohol with an oct-6-ene substitution at position 1 and methyl groups at positions 3 and 7. Cymbopogon possesses several pharmacological effects that have already been documented in the literature, including anticonvulsants, antihyperalgesic, and orofacial antinociceptive qualities [20][21][22]. β-Citronellol is a terpene whose class has previously been shown to possess a variety of beneficial pharmacological effects, so this study focused on reviewing the medicinal properties previously identified for β-citronellol that present the potential for treatments in humans. This was done in light of the need to find new sources of drugs that are more effective and have fewer side effects.

Molecular Formula: $C_{10}H_{20}O$

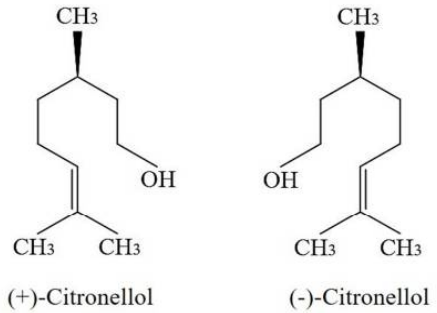


Figure 1: The 2-D dimensional image of β-Citronellol.

MATERIAL AND METHODS

The ethyl acetate extract of the leaves of *Ficus racemosa* was taken for the study [23]. Further, the extract was subjected to primary and secondary column chromatography of which pure compounds were extracted. For the isolation of phytoconstituents, silica gel column chromatography was used. The apparatus used is a vertical glass column made of borosilicate material. At the bottom of the column, a piece of wool was placed. On top of the glass wool, sand was added. Silica gel slurry was prepared by taking 200g of the gel, mixed with hexane, and poured from the top of the column. 13g of the extract was mixed with hexane and 5g of silica gel and poured from the top of the column along the sides, this was followed by pouring the solvent.

A systematic gradient elution method was followed to separate the fractions from the ethyl acetate extract. The column was first eluted with petroleum ether, ether: chloroform, chloroform: methanol,

methanol: chloroform, and chloroform. The flow rate was regulated to 5ml/min and for each of the fraction, 40 ml solvent was collected [24].

The fractions collected separately were concentrated and run on TLC to identify the presence of phytocompounds. The TLC used is aluminum sheets coated with silica gel 60 F254. For each spot, the Rf value was calculated.

Based on their bioactivity the promising compound was further analyzed for Spectroscopic studies- FTIR, and LCMS. The anti-bacterial studies were carried out and among these four compounds, only one compound showed good results.

Bacterial culture and Media - The soybean casein digest agar/broth of Hi Media was utilized for the antibacterial test. The bacteria were added to soybean casein digest agar broth, inoculated, and incubated for 4 hours at 370 C. The suspension was then examined to see whether it contained around 105 CFU/ml. Gram-positive *Staphylococcus aureus* (ATCC 7443) and gram-negative *Pseudomonas aeruginosa* (ATCC 7903) bacteria are the strains employed in the test. From cultures cultured on Tryptic Soya Broth (TSB) for bacteria for 16–18 hours at 37°C, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cell suspensions were made. Using the 0.5 McFarland Standard, cell density is adjusted to 1 x 106 cells per milliliter.

Pseudomonas aeruginosa and *Staphylococcus aureus* were inoculated on SCDA (Soybean casein digest agar) plates that were 90 mm in diameter. Test substances: Samples (10 ml) and (5 ml) of the common antibiotic Ciprofloxacin (concentration 10 ml) were introduced to the 5 mm well on agar plates for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The treated plates were incubated aerobically (HiMedia) at 35°C for 24 hours with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. We looked for an inhibitory zone surrounding the wells on the plates. The diameter of the observed zone of inhibition was used to gauge the antibacterial activity.

RESULTS

The Rf values and yield percentage were calculated for the 4 compounds that were isolated. Fig 1 shows the TLC plate of one of the compounds isolated. Based on the bioactivity of the compounds, one pure compound showed good results when compared to the others. The compound was taken for further identification and analysis. For the compound identification, Liquid Chromatography- Mass spectroscopy was done to know the different peaks present in the compound, The chromatogram shows the retention time and area (%) of the peaks. with a single major peak at a retention time of 8.959 min, shown in Figure 1. The structure of the pure compound was elucidated using FTIR analysis. The functional group C-O present in the compound was identified using Fourier-transform infrared (FTIR) spectroscopy, shown in figure 2, and the Mass-spectrum of the pure compound is shown in figure 3. Combing this spectral data, the structure of the pure compound was identified as β -citronellol, and the molecular formula of the compound is $C_{10}H_{20}O$.

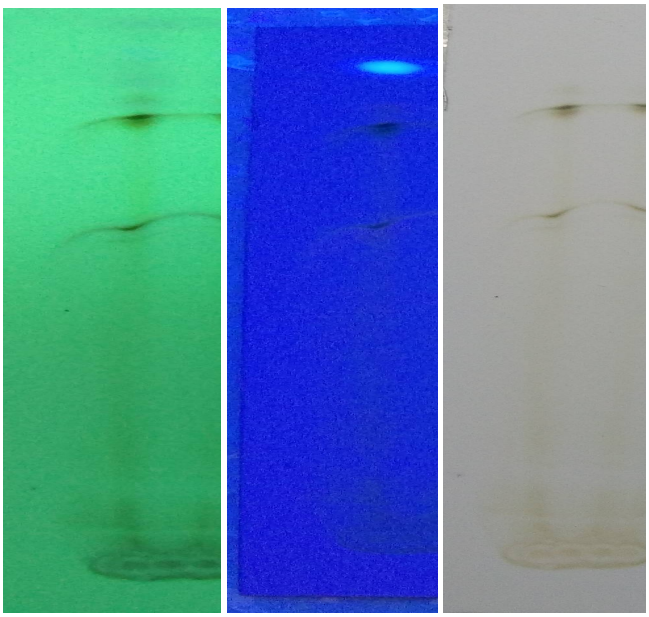
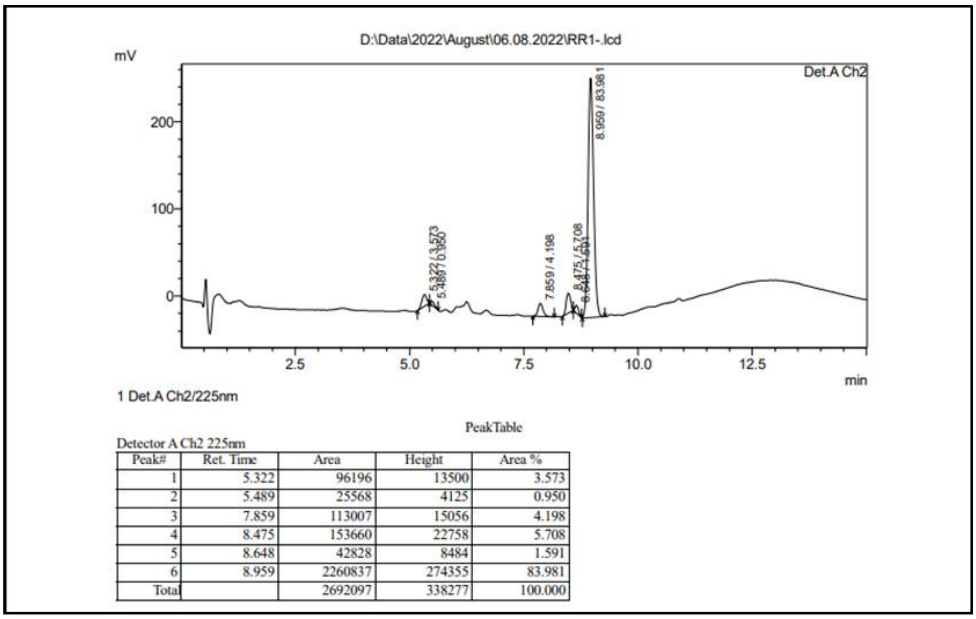


Fig1: TLC plate of one pure compound



PeakTable

Detector A Ch2 225nm

Peak#	Ret. Time	Area	Height	Area %
1	5.322	96196	13500	3.573
2	5.489	25568	4125	0.950
3	7.859	113007	15056	4.198
4	8.475	153660	22758	5.708
5	8.648	42828	8484	1.591
6	8.959	2260837	274355	83.981
Total		2692097	338277	100.000

Fig 2: LC-MS spectra of the compound.

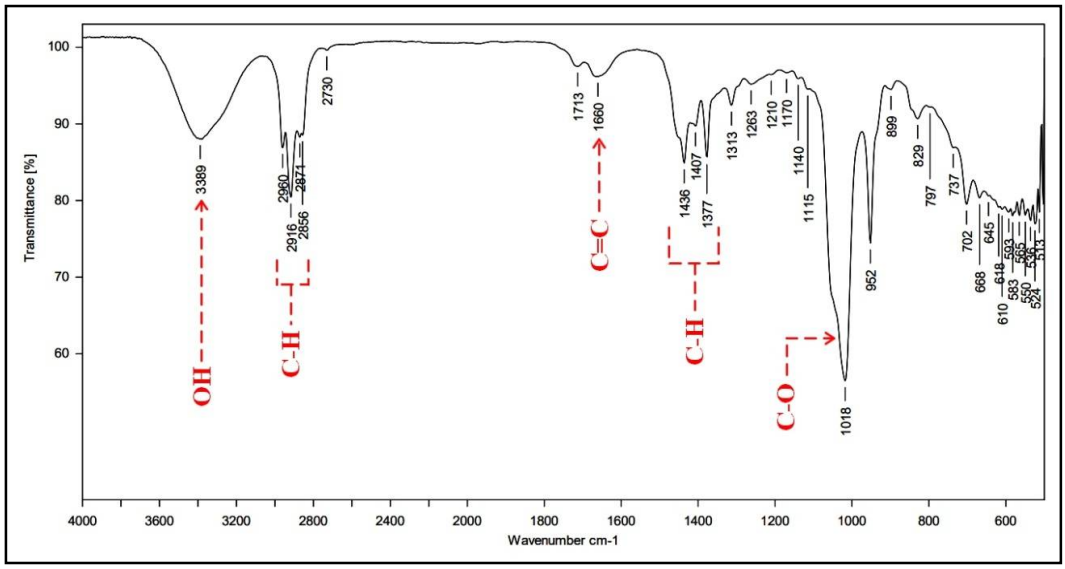


Fig 3: FTIR spectroscopy to know the functional group of the compound.

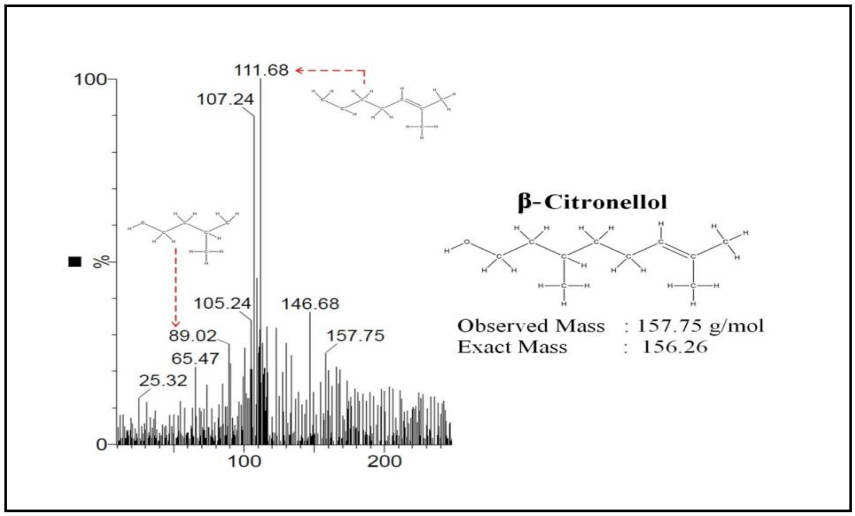


Fig 4: NMR the structural elucidation of the compound.

Anti-bacterial study

Results of the inhibition zone values for standard (Ciprofloxacin) and Sample (β-citronellol) against *S. aureus* and *P. aeruginosa* are presented in Figure 2 and Figure 3, respectively, and Table 1. According to the results obtained, the standard (Ciprofloxacin) showed high antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*P. aureus*) bacteria. Although the sample (β-citronellol) also showed a minimum zone of inhibition, not as high as compared to standard (Ciprofloxacin), resulting in the antimicrobial activity of the sample (β-citronellol).

Table 1: Inhibitory activity of test compounds against test organism

Test Sample	Test organism	Conc. Per well	Zone of inhibition (mm)
Sample (Ciprofloxacin)	*Staphylococcus aureus*	1 µg	15
Control (water)		10 µl	-
Sample (β-Citronellol)		5 µl	6
		10 µl	8
Sample (Ciprofloxacin)	*Pseudomonas aeruginosa*	1 µg	19
Control (water)		10 µl	-
Sample (β-Citronellol)		5 µl	6
		10 µl	9

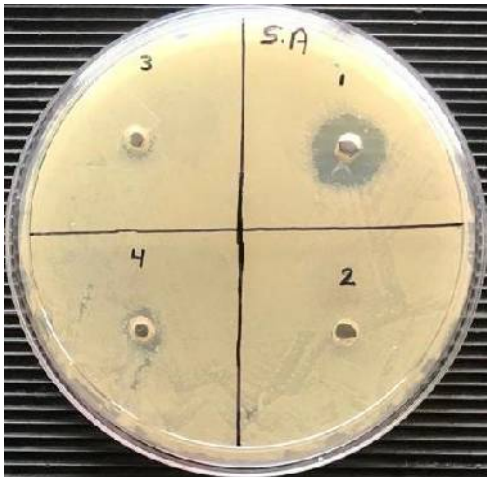


Fig-5: 1- Standard (Ciprofloxacin; 1µg/well), 2- Control (Water), 3- Sample (n=1; 5µl/well), 4-Sample (n=2; 10µl/well).

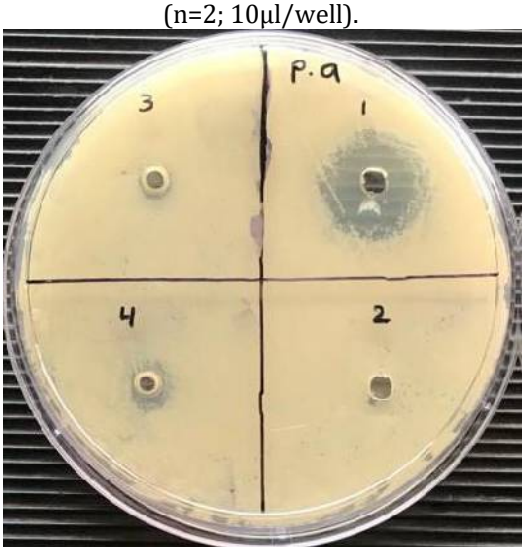


Fig-6: S- Standard (Ciprofloxacin; 1µg/well),2- Control (Water), 3- Sample (n=1; 5µl/well), 4-Sample (n=2; 10µl/well).

DISCUSSION

The findings of the study demonstrate that β-citronellol, an extracted molecule, has antibacterial action against gram-positive as well as gram-negative bacteria. The bactericidal activity of CT was initially studied by Kotan et al. in 2007 [25]. The effect of CT on various Gram-positive and negative species with clinical relevance, including *Staphylococcus aureus* ATCC 29213, *Streptococcus pyogenes* ATCC 176, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella typhimurium*, was described by the authors using a concentration of 30 mg/ml in the disc infusion method. The bacteria *Proteus mirabilis*, which causes urinary tract infections, was used by Echeverrigarav et al. to demonstrate the antibacterial efficacy of CT at a lower dosage (Minimal inhibitory concentration - MIC = 3 mg/l) [26]. Other studies priory stated the cytotoxicity [27], antibacterial [28], and analgesic-like activity in mice [29].

Terpenes and their derivatives, halogenated monoterpenes, are seaweed-derived chemicals that are often secreted outside of cells to protect against environmental stress and have strong anti-cancer properties. First monoterpene identified from *Portieria hornemannii* was the halogenated monoterpene halomon [6(R)-bromo-3(S)-bromomethyl)-7-methyl-2,3,7-trichloro-1-octene] [30] has sub-micromolar activity (IC50 0.9 M) against at least one cancer cell line, including solid tumor cell lines obtained from the kidney, the brain, and the colon[31]. When compared to the well-known anti-cancer medication cisplatin, other halogenated monoterpenes derived from the red seaweeds *Plocamium sure* as well as *Plocamium cornutum* showed better antiproliferative action on an esophageal cancer cell line (WHCO1) [32].

Both gram-positive and gram-negative bacteria were susceptible to the substance's antibacterial effects. Since the molecule is readily accessible from several plant sources, the study concludes that it has antibacterial capabilities and calls for additional research into the substance's possible bioactivities.

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