

# Anti-Cancer and Antimicrobial Activity of Novel Bacterium *Brevibacterium* Sp. from Saudi Arabia Peninsula

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## ABSTRACT

Widespread use of antibiotics is leading to wide spread of multi drug resistance posing a global burden to the health care sector. Species of *Brevibacterium* has been known having potential pharmaceutical importance mainly targeting cancer and other diseases. Pharma giants are now focusing on the terrestrial environment to isolate important medical compounds embedded in this natural ecosystem. Marine samples were collected from UQAIR region and tested for anti-cancer and microbial activity using standard protocols. The results shows that isolated stains have potential anti-cancer and antimicrobial activity and strains have been identified by 16s ribosomal DNA (rDNA) sequence. The antibacterial activity of ethyl acetate crude extracts at the concentration of 10 mg/well was assayed by agar well diffusion method against pathogens- *Staphylococcus aureus* (ATCC-29213) and *E. coli* (ATCC 25922). The results obtained from in vitro cytotoxicity study of MCF-7 (IC50-17.3), HeLa (IC50-26.2) and HCT-116 (IC50-29) have optimal anticancer activity comparing to standard. In conclusion, the bacteria isolated from sea water showing promising results and active bioactive compounds isolated from marine atmosphere may be used to explore the active molecules have the potency of anti-microbial and anticancer activity.

**Key words:** *Brevibacterium* Sp, Bioactive compound, Anti-cancer, Antimicrobial, RT-PCR

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## INTRODUCTION

Complementary and Alternative medicine are emerging as an alternative support for traditional methods for many cancer and other various types of human diseases [1]. The pharma giants are now focusing on the terrestrial environment to isolate important medical compounds embedded in this natural ecosystem [2]. Ecosystem sustainability is one of the natural phenomenon where microorganisms paly crucial role in regulation concerning environmental changes [3]. The repeated failure of antibiotic resistance and chemotherapeutics agents has led to screening of novel compounds from various natural resources for their optimal anti-cancer and antimicrobial components. Today, many human diseases been treated with natural remedies which may be rich source of natural antimicrobial anti-cancer and other activate components [4,5]. Natural products possibly crude forms provide great opportunity for many researchers to identify active compounds may give lead in novel drug discovery. Nowadays, trends in research focusing their attaining to folk medicine may have some reasonable answers to many synergetic diseases.

Marine biota inhabit rich in compounds with unique medical prosperities [6], approximately more than 230 bioactive natural products were identified from previous studies out and out 102 compounds shown potential antimicrobial and anticancer activity [7].

In our current project we are isolating effective microbial flora from marine atmosphere may have potential outcome in encountering various pathogens, subsequent studies have postulated and convincing that most of the antibiotics we use in day today life originates from natural sources [8], so led the path to focus on the marine and bioactive compounds [9]. Regular search for bioactive compounds is must now a day, one of the reasons is economic burden and due to several aspects of multi drug resistance posing a huge threat to the human welfare. The aim of the present study is to extract the crude by solvent extraction next step by identification of bacterial species from marine samples collected from UQAIR beach area located in the Eastern Province, Saudi Arabia and to evaluate anti-cancer and microbial activity of the crude extract isolate

## MATERIALS AND METHODS

### Collection of marine

Marine Samples like soil and marine water were

collected from various parts of coastal areas. Later samples were brought to laboratory with the help of ice and refrigerated for the analysis.

### Isolation of bacteria

The pure strains of marine bacteria were isolated from the collected marine samples. 1.0 ml amounts of each marine sample were taken in a series of 50 ml of Zobell broth and incubated at 35°C for 24 hours at 150 rpm in orbital shaker. After incubation one loopful of each bacterial culture broth was inoculated on the marine Zobell agar plates and incubated in 25°C for 18 hours. The bacterial colonies obtained were sub-cultured on fresh slants.

### Screening of antibiotic producing bacteria

The isolated strains were then screened for antimicrobial activity against standard clinical isolates; *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*. The screening of potential strains was done by cross streak method as described [10].

### Identification of bacteria

The Amplification of the 16s ribosomal DNA (rDNA) sequence of strain was carried out using amplification primers forward (5'-CTYAAAKRAATTGRCGGRRSSC-3' and reverse (5'-CGGGCGGTGTGTRCAARRSSC-3'; using the procedure described. BLAST programme used to analyze the resulting sequencing and compared with the database Genbank from NCBI.

### Crude extraction for bioactive compounds

The isolates inoculated in to Zobell broth and incubated in suitable conditions. Max production of antibiotic observed after 24 h incubation and biomass was obtained after termination of growth on 8th day after broth centrifuged for 160 min at 9,000 rpm. Extraction of the crude was initiated by using various solvents as methanol and ethyl acetate used at 1:1 ratio. The mixture was agitated with homogenized for 45 min and the solvent was separated from broth using funnel. The supernatant was pooled and filtered through crude filter paper, followed by the whatman No.1 filter paper and then it was concentrated using a speed vacuum concentrator (Speed Vac, Rplus C 210 A, Savant). Weight of this crude extract was determined using an electronic balance (Infra Tech, model: IN200). This crude extract was used for further analysis.

### Antibacterial activity

Agar diffusion method is adopted same as in many studies to estimate the antimicrobial activity of the crude extract obtained from solvent extraction. 15-20 ml Mueller Hinton agar plates were prepared and allowed to solidify, culture strains to be tested were evenly spread on media plates using cotton swab and 0.6 cm wells on the plates were made using sterile borer. 100 ml

of prepared crude extract were poured in each well and later plates were incubated at 37°C for 24 h. Tetracycline used as a stander and media as a blank for the test, after incubation results were interpreted by measuring the zone of inhibition.

### Anticancer activity

Cell culture: MCF 7, HeLa and HCT 116 cell lines were cultures in DMEM media (GIBCO-Thermo) with 10% FBS (GIBCO-Thermo) and 50 µg/mL gentamicin and the culture incubated at 37°C in CO<sub>2</sub> incubator, humidifying with 5% CO<sub>2</sub>. The cells were subculture until the cells in the exponential phase later these cells were sued for MTT assay.

### Cell viability by MTT assay

Cytotoxic effect of the extract (UQAIR 1) was determined by MTT assay. In brief exponentially grown cell culture was considered for the assay. Cells were seeded in 96-well plate with 100 µL of media at a concentration of 104 cells/well. Extract in 0.1% DMSO (Thermo-USA) was serially diluted with media to obtain appropriate concentration and incubated for 48 h along with media serving as control. After incubation, the cells were washed with PBS followed by adding 200 µL of MTT reagent further cells were incubated at 37°C for 4 h, later media from the wells was washed out and 100 µL DMSO added to each well and absorbance was measured at 540 nm using microplate reader (Biotech), each test was taken in triplicates using doxorubicin as standard and 0.1% DMSO as a blank.

### Detection of cell death

The effect of crude to induce cell death in MCF-7 cells was determined by AO/EtBr dual staining. The cells were grown on the cover slip in 24-well plate with 1 × 10<sup>5</sup> cells/well, then the cells were treated with the IC 50 (80 µM) concentration of the compound for 24 h. After incubation, 5 µL of AO (1 mg/mL) and 5 µL of EtBr (1 mg/mL) were added, and the induction of cell death was observed by using a fluorescence microscope.

## RESULTS

In the present research investigation, the selected water and soil samples were collected from two locations of UQAIR beach area located at located in the Eastern Province, Saudi Arabia for isolation of bacteria. The successful isolation of bacteria from water samples were found among the 76 bacterial colonies found in two locations 16 colonies were shown unique in nature and rest all the colonies are found to be similar in morphology. All the 16 selected strains were initially screened to identify their capacity to produce antimicrobial compounds. Among the strains, UQAIR 1 showed strong antimicrobial activity against the *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* tested pathogens (Table 1).



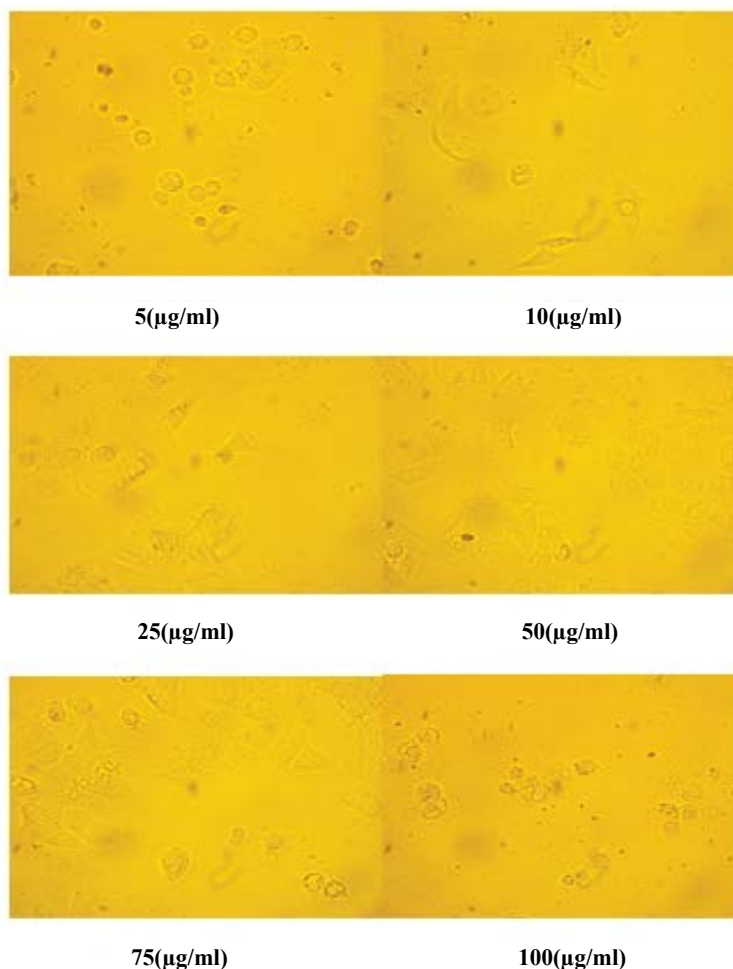


Figure 2: Anticancer activity of crude extract of *Brevibacterium* sp UQAIR 1 strain.

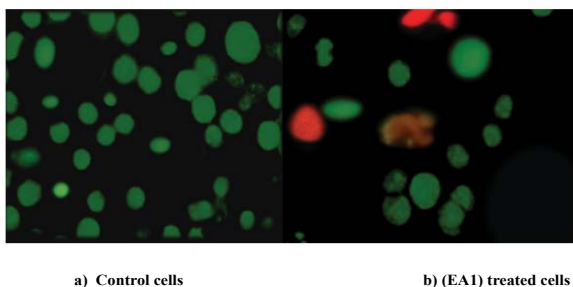


Figure 3: AO/EtBr staining of MCF-7 cells a) Control cells b) (EA1) treated cells

3). The EA1 extract to induce cell death in the MCF-7 cells was proved with the results of AO/EtBr staining.

**DISCUSSIONS**

An antibiotic is a substance like a chemical produced naturally by microorganisms, which has the capacity to inhibit the growth pathogenic microorganisms in low concentration [11]. Antibiotics have remained unrivaled in their control of infectious microorganisms for the past 60 years. Present research is focuses on the discovery of new or novel antibiotics and anti-cancer compounds and further modifications to existing antibiotics are

still in process by all researchers for the discovery and development of antibiotics with novel structural classes are particularly important, including the development of resistant bacteria and other pathogens [12]. Many technologies that have been successfully applied to terrestrial microorganisms can be applied to marine microorganisms in order to achieve overproduction of the desired product. Random mutagenesis and selection techniques are common for strain improvement [13]. Many recent studies biomedical field in priority targeting microbial genera like bacteria and fungi producing novel metabolites, coastal flora have been reported to yield antitumor, anti-inflammatory and antibiotic compounds [14-16], these symbiotic also proven to be a rich sources of bioactive metabolites and compounds with pharmaceutical importance. Marine sphere which is also emended with bacterial and fungal species capable of producing many bioactive compounds having optimal impact [17,18].

**Antibacterial activity**

In our present investigation, out of 76 isolates, UQAIR 1 showed strong antimicrobial activity against the *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* tested

pathogens, further crude extract cultured in 1000 ml conical flask and the bioactive metabolite was extracted. The ethyl acetate (EA1) extract has shown potential antimicrobial activity against tested pathogens. Similar results have been reported where marine *Alteromonas rava* shown to be effective against Gram negative and positive bacteria [19,20].

### Anticancer activity

In the recent advancement of searching alternative medicine for anticancer cancer and antimicrobial activity, marine flora have shown successful bioactivity compounds and sore of secondary metabolites has the ability to encounter against various infectious diseases [21]. Our results have shown productive anti-cancer activity tested compared to standard as these can be related for further analysis which can give an edge in novel drug compounds. It is significant that marine sources have also demonstrated tremendous abilities as producers of anti-cancer compounds and secondary metabolites that act against infectious diseases and inflammation [22]. Previous studies have added with evidence that out of 4196 marine compounds isolated 56% shown anti-cancer action and 13% effective antimicrobial activity which can be assume that marine compound has great privilege in alternative medicine [23].

### CONCLUSION

In conclusion, the bacteria isolated from seawater showing promising results having active bioactive compounds may be used to explore the active molecules have the potency of anti-microbial and anticancer activity. From our current study, alcoholic extracts has optimal anticancer and antimicrobial activity which need further studies to elevate the current results.

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