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Investigation on pharmacological activities of secondary metabolite extracted from a mangrove associated actinobacterium *Streptomyces olivaceus* (MSU3)

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ABSTRACT

The present study was undertaken to evaluate pharmacological activities of secondary metabolite of *Streptomyces olivaceus* (MSU3) isolated from the sediment sample of a mangrove ecosystem. The isolated strain was screened for its preliminary antagonistic property against various clinical pathogens and its secondary metabolite was extracted by using ethyl acetate. The 30 μl concentration of the crude extract of the isolate expressed the maximum zone of inhibition of 27 ± 2.44 mm against the bacterial pathogen *Streptococcus mutant* with the MIC and MBC values of 0.625 and ≤ 5 μg/ml concentrations, respectively. Further different concentrations of extract were tested for *in-vitro* antioxidant, antiinflammatory and *in-vivo* cytotoxicity studies. It expressed the maximum percentage of *in-vitro* total antioxidant activity of 87%, DPPH scavenging activity of 62.06%, reducing power effect of 32.51%, hydroxyl radical scavenging activity of 47.99% and nitric oxide activity of 33.20% at 100 μg/ml concentration respectively. Further the extract exhibited 96.63% of inhibition of *in-vitro* antiinflammatory activity, 49.60% of total hemolytic activity and also has 42.11% of total phenolic content at respective concentration of 500 μg/ml. The cytotoxic effect of the crude extract was analyzed by MTT assay on MCF-7 and HT-29 cell lines and the cell viabilities were observed as 24.00% and 39.17% at 250 μg/ml concentration with the respective IC_{50} values of 88.26 and 104.81 μg/ml. From the results, it is evident that the ethyl acetate crude extract of *S. olivaceus* (MSU3) has potent antimicrobial, antioxidant, antiinflammatory and cytotoxic activity and suggested that the isolated strain could be a candidate for the nature resource of pharmaceutical.

1. Introduction

Free radicals are highly reactive particles produced by the body either as a by-product during normal biochemical process like enzyme activation. Under normal condition, the body is capable of neutralizing these particles and maintains them at a safe minimum level. Excess or abnormal formation of free radicals is potentially dangerous and can lead to oxidation and even irreversible damage of body tissues (Sato et al., 1996; Badmus et al., 2011). An antioxidant acts as a free radical scavenger and neutralizes these reactive particles by binding to their free electrons. By destroying free radicals, antioxidants help to detoxify and protect the vital body tissues and organs. Cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effect (Sato et al.,1996). These mechanisms are not sufficient when the balance shifts to the side of free radical generation, thus body requires antioxidant supplements to reduce oxidative damage. Antioxidants are molecules capable of preventing oxidative damage. Recent investigation suggested that antioxidant capacity of putative antioxidants can be attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging in body cells alleviating lipid, protein oxidation and may reduce potential mutations (Sheikh et al., 2009). Therefore the antioxidants help to prevent degenerative diseases and other pathologies (Gautham and Onkarappa, 2013). Basically there are two types of antioxidants, as natural and synthetic antioxidants. Many studies reported that the synthetic antioxidants are suspected to have carcinogenic probability; therefore, natural antioxidants are the most preferred type (Mousumi and Dayanand, 2013). Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Antioxidants therefore are used to reverse the

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harmful effects of the free radicals by scavenging the free radicals and detoxifying the physiological system (Farnet et al., 2005; Gautham and Onkarappa, 2013).

Microbial based natural products are notable not only for their potent therapeutic activities, but also for the fact that they frequently possess the desirable pharmacokinetic properties required for clinical development; many microbial natural products reach market without any chemical modifications, a testimony to the remarkable ability of microorganisms to produce drug like small molecules (Farnet et al., 2005). Members of the actinomycetes genus, especially *Streptomyces* sp. have been recognized as prolific producer of useful bioactive compounds with broad spectrum of activities that produce about 75% of commercially and medically useful antibiotics (Moncheva et al., 2002). Among various genera of actinomycetes, *Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora* and *Actinoplanes* are the major producers of secondary metabolites (Karthik et al., 2011). These secondary metabolites produced by actinomycetes have a broad spectrum of biological activities (Karthik et al., 2013). *Streptomycetes* and related actinomycetes continued to be useful sources of novel secondary metabolites with a range of biological activities that may ultimately find applications as anti-infective, anti-cancer agents or other pharmaceutically useful compounds (Forar et al., 2006). The metabolites like benthocyanins and carquinostatin produced by *S. prunicolor* and *S. exfoliates* showed maximum antioxidant activity (Karthik et al., 2013). *S. chibaensis* AUBN1/7 (Gorajana et al., 2007) and *Streptomyces* spp. KR-5 exhibited cytotoxic activity against the growth of human breast cancer cell line (Sateesh et al., 2011). Whereas, *Streptomyces* spp. SRDP-H03 (Rakesh et al., 2013) and BI244 (Kiruthika et al., 2013) exhibited both anti-inflammatory and antioxidant activities. Considering the importance of the above, the present study was focused on the antimicrobial, antioxidant, anti-inflammatory and cytotoxic properties of secondary metabolite extracted from a novel actinobacterium *Streptomyces olivaceus* (MSU3) isolated from the rhizosphere sediment samples of Manakudy estuary, Kanyakumari District, South India.

2. Materials and methods

2.1. Sample collection

The culture *S. olivaceus* (MSU3) was isolated from rhizosphere soil of mangrove plant *Rhizophora mucronata* of Manakudy estuary, Kanyakumari District, South India and it was identified and confirmed up to species level through 16S rRNA partial sequencing method described by Nathan et al. (2004). The isolated strain was sub-cultured in sterile ISP2 media (Yeast extract-Malt extract agar) and incubated at 28 °C for 7 days. After incubation, the isolate was kept in MNP laboratory at Centre for Marine Science and Technology, M.S. University, as reference strain and it was used as a working strain for further studies.

2.2. Test organisms

The clinical pathogens used in this study were *Streptococcus mutant* (NCIM2063), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae (*ATCC10273), *Streptococcus pneumoniae* (ATCC49619) and *Vibrio cholerae* (MTCC3905) obtained from MNP laboratory of Centre for Marine Science and Technology, M.S. University.

2.3. Preliminary screening of antagonistic activity of S. olivaceus (MSU3) through cross streak method

The antagonistic activity of the selected actinobacterial strain *S. olivaceus* (MSU3) was screened by using cross streak method (Devi et al., 2012; Alexander et al., 1977). The isolated strain was streaked as a straight line at the middle of the petriplate containing Modified nutrient glucose agar (NA + 1% Glucose) medium (MNGA). After inoculation, the plate was incubated at 28 °C for 7 days for their growth. After incubation, 24 h old pathogenic bacterial strains were individually inoculated perpendicular to the growth line of selected actinobacterium in the same plate. The cross streaked plate was further incubated at 37 °C for 24–48 h and the extent of inhibition was observed. The absence of growth or a less dense growth of test bacteria near the actinobacterial isolate was considered positive for production and secretion of antibacterial metabolite by the isolate.

2.4. Extraction of secondary metabolite from S. olivaceus (MSU3)

The secondary metabolite of *S. olivaceus* (MSU3) was extracted by using the method described by Zhong et al. (2011). The spore suspension of the actinobacterium *S. olivaceus* (MSU3) was inoculated into the ISP2 agar plates and incubated at 28 °C for 7 days. Then the aerial mycelia were scraped and soaked in 80% ethyl acetate for 24 h. It was centrifuged (4000 rpm, 10 min) and extracted twice more. The supernatant was dried and dissolved with distilled water and then it was extracted with three half volume of ethyl acetate. The crude ethyl acetate extract was dried in vacuum at 40 °C and the dried powder was used for further studies.

2.5. Secondary screening of antagonistic activity of the crude extract of S. olivaceus (MSU3) through agar well diffusion method

Antibacterial activity of the crude extract of *S. olivaceus* (MSU3) was determined by agar well diffusion method (Kekuda et al., 2012; Rakesh et al., 2013). Wells of 5 mm diameter were made using sterilized cork borer on Mueller Hinton Agar. 0.1 ml each of inoculum of test pathogens were spread on the plates and different concentrations (10–30 μl) of the crude extract was tested for their activity against the test pathogens and chloramphenicol (25 μg/ml) was used as positive control. Plates were incubated at 37 °C for 24 h and the zone of inhibition was measured.

2.6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the extract against the individual test pathogens was determined by using the method of Akinjogunla et al. (2010) with slight modification. 2 ml of sterile nutrient broth with different concentrations of the extract (0.313, 0.625, 1.25, 2.5, 5, 10 and 20 μg/ml) were taken in test tubes and to this 0.1 ml each of test pathogens were added. Then the test tubes were incubated at 37 °C for 24 h. Similar test tube sets containing chloramphenicol at the concentration of 25 μg/ml were used as control. After incubation, the tubes were examined for microbial growth by observing the turbidity. The tubes containing the least concentration of extract showing no visible sign of growth was considered as the minimum inhibitory concentration. To determine the MBC of the extract, 0.1 ml each of the culture broth was collected and inoculated onto sterile nutrient agar. The plates were then incubated at 37 °C for 24 h. After incubation, the lowest concentration yielding negative subculture was considered as Minimum Bactericidal Concentration (MBC). Both MIC and MBC for the test bacteria were determined in triplicate assays.

2.7. Determination of total phenolic content

The total phenolic content of the crude extract of *S. olivaceus* (MSU3) was determined by employing Folin–Ciocalteu Reagent