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BIOPROSPECTING ENDOPHYTIC FUNGUS FROM ALEO VERA AND EXPRESSING THE HOST COMPOUNDS IN ISOLATED CULTURES.

H. Jayasudha[1], V. Hemamalini[2], Fathaunnisha. S[3], M. Menaga

R. Sridhar[5]

[1,3]Research Scholar, Department of Plant Biology and Plant Biotechnology, Quaid-E-Millath College for women, Chennai.

[2]Assistant Professor, Department of Plant Biology and Plant Biotechnology, Quaid-E-Millath College for women, Chennai.

[4] Managing Director, Bioneemtec India, Pvt.ltd. Siruseri, Chennai.

[5]Research Scholar, Department of Education, Tamil Nadu Open University, Chennai

Abstract

Medicinal plants and their endophytes are potent resources for discovery of novel bioactive metabolites. The endophytic fungi from plants were an important source for the production of various secondary metabolites, bioactive compounds which are useful in pharmacology, agriculture and in industries. Endophytic fungi are a group of fungi that colonize living and internal tissues of plants without causing any immediate, over negative effects The capability of medicinal plant endophytic fungi for the production of bioactive secondary metabolites was evaluated under in vitro conditions. In the present investigation, the diversity of endophytic fungi was studied associated with medicinally important plant *Aloe vera*. A total of 50 fungal species from Aloe vera were isolated and categorized under eight species based on the morphology of the fungal culture and characteristics of the spores. Secondary metabolites produced from isolated endophytic fungi was subjected to investigation and challenged for their antibacterial activity. Among the eight endophytic culture isolated , all the cultures were subjected to medium optimization to produce host marker compounds . The crude extract produced was profiled using thin layer chromatography and found that *Aloe vera* host compounds were also found secreted by the isolated endophytic fungus . Thus it gives an interesting note that medicinally important *Aloevera* compounds shall also obtained from the endophytic cultures isolated from aloevera , with out exploiting the *Aloe vera* for its compounds .

Keywords: Endophytic fungus, Aloe vera, Secondary metabolites, Antibacterial activity

INTRODUCTION

Endophytes are microbes (fungi/bacteria) that live within the plant tissues (leaves, stem, roots) without causing any noticeable symptoms of disease.[1] The most frequently isolated endophytes are fungi. The term "endophytic fungi" refers to an organism which lives within plant tissue by forming symbiotic relationship with host [2]. Plant endophytic fungi have been found in each plant species examined, and it is estimated that there are over one million fungal endophytes existed in the nature [3]. Plant endophytic fungi have been recognized as an important and novel resource of natural bioactive products with potential application in agriculture, medicine and food industry [4-6]

Aloe vera (L.) Burm.f is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall [7]. Commonly referred to as Aloe vera, is one of more than 400 species of Aloe belonging to family Liliaceae [8]. The species does not have any naturally occurring populations, although closely related aloes do occur in Northern Africa [9]. It is a cactus-like plant that grows readily in hot, dry climates and currently because of demand; it is cultivated in large quantities [10]. Aloe vera plant almost sessile perennial herb, has leaves 30-35cmm long and 10cm broad at the base, colour pea-green (when young), bright yellow tubular flowers 25-35cm in length arranged in a slender loose spike, stamens frequently projected beyond the perianth tube [11]. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20µg/ml of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources [12]. The skin plays an important role in protection from the body internal environment and it is the largest organ in human's body so exertion of serious damage to this organ may cause several problems in its survival [13]. Medicinal plants according to the World Health Organization (WHO) defines them as herbal preparations made by introducing plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes, which may be produced as a basis for herbal products or for immediate consumption [14]. Aloe vera has modified thick fleshy leaves, it not only has cell wall carbohydrates such as cellulose and hemicellulose but also storage carbohydrates such as acetylated mannans the polysaccharides found in the inner leaves parenchymatous tissue have medicinal importance and also the biological activities are due to presence of large number of compounds [15]. The herb is used internally to combat most digestive problems, including constipation, poor appetite, colitis, irritable bowel syndrome as well as, asthma, diabetes, immune system enhancement, peptic ulcers. Aloe is used externally for the treatment of skin irritation, burns, scalds, sunburn wounds, eczema, psoriasis, acne, dermatitis, ulcers, to stimulate cell regeneration. The plant is also used in the treatment of healing properties, effects on skin exposure to UV and gamma radiation, anti-inflammatory, antiviral and antitumor, moisturizing, anti-aging effect, antiseptic, enhance immune system, hypoglycemic, cytotoxic, and anti-diabetic effects, antibacterial effect, antioxidant, cardiovascular effect [16]. Its juice may help some people with ulcerative colitis, an inflammatory bowel disease [17].

2. MATERIAL AND METHODS

2.1. Collection of Plant Sample

The *Aloe vera leaves* were collected from women Biotech Park, Sipcot industrial estate, Siruseri, Chennai. The leaves of the Superior portion of the plant (20 cm above the soil) were collected

2.2. Isolation of Endophytic Fungus

Aloe vera leaves were collected and washed with the running tap water to remove soil particles. The cleaned leaves were surface sterilized by immersion in 70% ethanol for 1 minute, followed by 2.5% sodium hypochlorite solution for 5 minutes, ethanol for 30 sec and then washed thrice (1 minute each time) with sterile distilled water and left for drying under sterilized condition. The small leaf segments were placed on potato dextrose agar (PDA)[18] media plates supplemented with antibiotic streptomycin (100mg/ml) to avoid bacterial growth. Each plate was inoculated with seven leaf segments. All the plates were sealed and packed with parafilm and were incubated at 18ºC to 28ºC. The plates were observed daily for 15-20 days for the emergence of endophytes. The emerging fungal hyphal tips from the plant leaf segments were picked and transferred on PDA plates to check the purity of the culture.

A fungal outgrowth from the plant tissues were sub-cultured on fresh antibiotic-free medium for identification established on morphological examination and conidial characters. All the colonies were counted and expressed as CFU per gram of fresh tissue. The pure cultures were maintained on PDA slants at 4°C.

2.3. Fermentation and Extraction

The PDA broth was prepared with all the optimized parameters with carbon sources, nitrogen sources, with prescribed pH. After prescribed duration the broth were taken and sacrificied. The broth was get filtered with whatmann filter paper to remove all the cell debris from the broth. The clarified broth was subjected to solvent extraction ethyl acetate to the broth double the amount of ethyl acetate was added kept stirring for four hours. After four hours ethyl acetate portion are separated from the water using separating funnel. This was done three times and all the ethyl acetate portion separated was clubbed together and distilled under rotor vaccum evaporation to get crude extract. The crude extract was run in the TLC with solvent proportion of hexane: ethyl acetate (1:1) and dipped in the vanillin reagent to observe the various bands present in the crude extract.

2.4. Inoculum Preparation

The fungal isolates used in this study was isolated from the plant *Aloe vera*. The isolates separated was evaluated for its antibacterial activity and the best one strain was taken for the maximum production of

secondary metabolites in broth in terms of yield and purity. The best isolates was cultivated on the potato dextrose broth medium using a 500ml Erlenmeyer flask at pH 6.5 and 37oC for 24 hr, after 24 hr of incubation a loopful of culure was taken in Erlenmeyer flask and inoculated on PDA agar slants and incubated at 37oC for 72 hr and used as inoculums.

2.5. Test Organism

The antibacterial assay of Aloe vera endophytic extract were determined using some clinical pathogenic microorganism. The test microbes such as Staphylococcus aureus(MTCCB 737), Escherichia coli (MTCCB 82), Bacillus(MTCCB 1272), Pseudomonas aurginosa (MTCCB 741) were obtained from Microbial Type culture collection, Pune, India.

2.6. Screening of Antibacterial activity

Antibacterial activity of fungal endophytic extract was determined using a modified Kirby Bauer Disc Diffusion Method[19]. The antibacterial activity was determined by the agar well-diffusion method on Nutrient agar (Hi Media, India) medium. Using a cork borer, wells (6 mm in diameter) were punched out in the agar medium and inocula containing 106cfu/mL of the each test bacteria were spread onto the surface of the medium with a sterile spreader. 50µl of the extract was pipette into the wells,. The agar plates were incubated at 37ºC for 24 h and the diameter of the zone of inhibition surrounding the wells was measured after incubation. The diameters of zone of inhibition due to extracts were compared with those produced by the commercial control antibiotics, Ampicillin (2mg/ml).Then 0.1g of the crude extract was dissolved in 10 ml DMSO (Di-methyl sulfoxide) to obtain the concentration of the extract. The active positive control were used Ampicillin 2mg/ml (2000 ppm parts per million). The antagonistic positive endophytic extract from *Aloe vera* (BNT 01, BNT 02) were taken for antibacterial screening assay. Antibacterial tests were performed in triplicates and observed values of zone of Inhibition were expressed as average value.

3. RESULT AND DISCUSSION

Antibacterial Activity of *Aloe vera* extract was evaluated against gram-positive and gram-negative bacteria. All the isolated strains showed an excellent inhibitory against clinical pathogens.

Among the test strain, crude extract of *Aloe vera* (BNT 02) have shown effective inhibition against all clinical pathogens. The extract of *Aloe vera* extract (BNT 01) displayed a moderate inhibition against all clinical pathogen.

In the last decade, *Aloe vera* has been used extensively in healthcare products including topical creams, cosmetics, and health drinks. All products available in the market claim for the beneficial properties based on the extensive research carried out across the world on different species of *Aloe* including its antimicrobial properties.

Our study results showed that *Aloe vera* (BNT O2) extract preparation had a stronger retardation effect on gram-positive test organisms (*S. aureus, and Bacillus)* as compared to the gram-negative bacteria (*E. coli, P. aurginosa*). Similarly, results were documented, in an earlier study where[19] the gram-positive test organisms were found to be more susceptible to the sterile *Aloe vera* gel preparation and the antimicrobial susceptibility testing of *Aloe vera* gel has a greatest inhibitory effect on the *S. aureus* with 12.0 mm diameter of zone of inhibition. Results of the present research also correlates with the earlier findings by Kaithwas *et al.* 2012[20] as well as studies conducted by where [21] it was demonstrated that the *Aloe vera* gel is rich in a wide variety of secondary metabolites, such as polysaccharides, anthraquinone, glycosides, glycoproteins, gamma-linolenic acid, prostaglandins which was found to be very effective against Gram-positive and particularly against *S. aureus*.

Table 1 - Antibacterial activity against crude extract of *Aloe vera* (BNT 01 AND BNT 02)

TEST PATHOGEN	BNT 01			BNT 02		
	500 ppm	1000 ppm	Control AMPICILLIN	500 ppm	1000 ppm	Control AMPICILLIN
E.Coli	No Activity	10mm	15mm	8mm	10mm	15mm
S.Aureus	No Activity	9mm	14mm	8mm	12mm	15mm
Bacillus	No Activity	9mm	15mm	9mm	12mm	15mm
P.Auruginosa	No Activity	10mm	15mm	10mm	11mm	15mm

Fig 1. Antibacterial activity of *Aloe vera* extracts BNT O1, BNT 02 was evaluated against gram- positive and gram-negative bacteria.

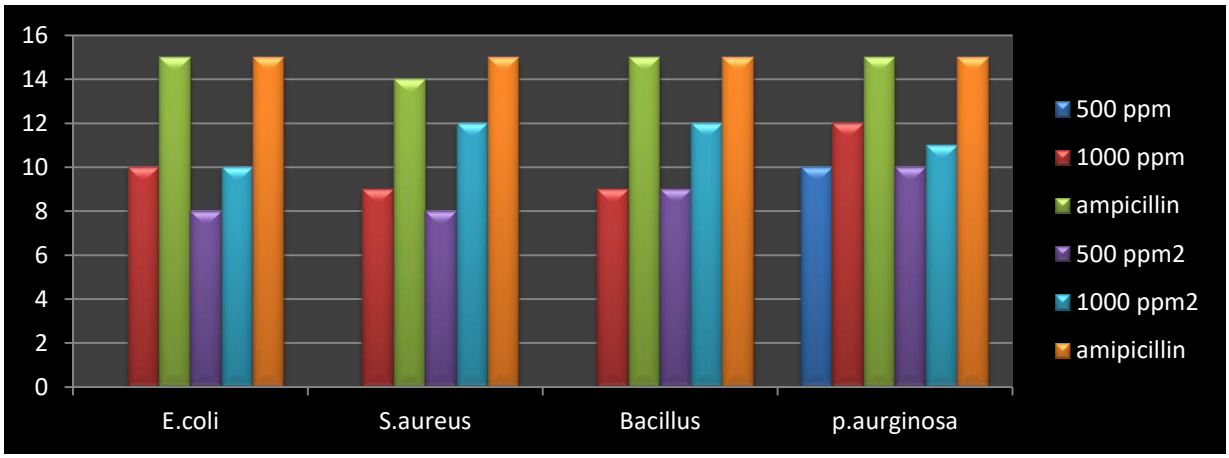


Fig 1. *Aloe vera* extract BNT O1 tested with E.coli, P.aurginosa , Bacillus, S.aurginosa

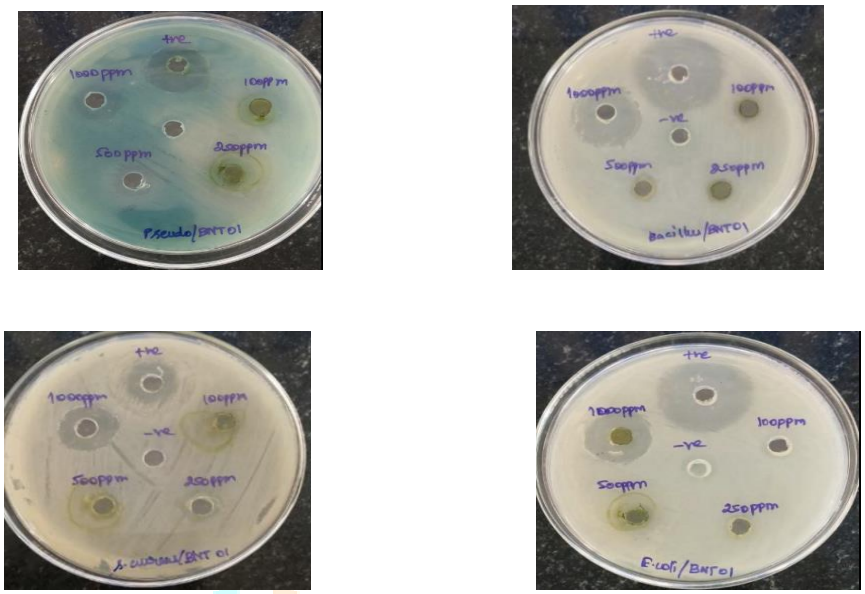
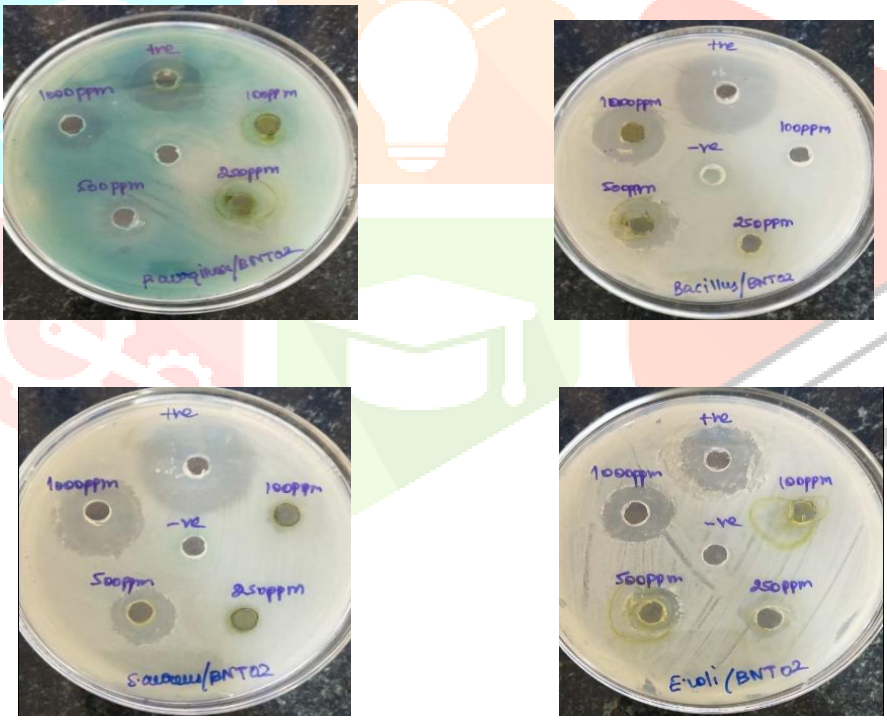


Fig 2. *Aloe vera* extract BNT O2 tested with E.coli, P.aurginosa, Bacillus, S.aurginosa



4. CONCLUSION

The present study has showed the possibility of the presence of some bioactive components in crude extracts of Aloe Vera due to which it has showed strong antibacterial activity. Moreover, the further analysis on bioactive components of Aloe Vera would be suggested by Column chromatography and structure elucidation with NMR,Mass spectrometry.

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