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Antimicrobial Activity and Production of Bioactive Compounds from Endophytic Bacteria of Tropical Cacti Plant Euphorbia Caducifolia

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Abstract - *Endophytes are the microorganisms that can reside within robust plant tissues by just having a symbiotic association. Main aims were to isolate endophytic bacteria and extraction of photochemical as secondary metabolites to explore abundances of endophytes. The endophytic bacteria from plants have an important role, such as a growth promoter with the production of plant growth regulators, besides that it can supply the plant nutrient and they are necessary for the growth and development of the plant. A plant and bacterium association between Euphorbia caducifolia and endophytic bacteria can produce secondary bioactive compounds which are helpful in defence activity from biotic and abiotic stress. Bioactive compounds are the natural molecules synthesized by plants and play a vital role in living beings as a antimicrobial, anti diabetic, anti cancer agents. Euphorbia caducifolia was from semi arid dry regions of banaskantha district Gujarat, India. Endophytic bacteria were isolated from root, stem and latex of plant E. caducifolia. Total ten isolated were obtaining from stem, root and latex of E. caducifolia.*

***Key Words*: *Euphorbia caducifolia*, Endophytic bacteria, Seconadry compounds, Photochemical, Antimicrobial activity.**

1. INTRODUCTION

All plants are important medically. The plant endosphere contains a diverse group of microbial communities. Endophytes are the microorganisms may be bacteria; fungi or actinomycetes .Endophytic organisms live within the plant tissues and can promote host species tolerance to different environmental stresses. Almost all plants species have been found to harbour endophytic bacteria or fungi (1) Sturz and Nowark, 2000).The term Endophytes was first coined by De Berry in 1866. They are also reported to supply essential vitamins to plants (2) Rodelas et al., 1993), confer protection against plant pathogenic microorganisms via production of antibiotics or synthesis of secondary metabolites (3) Long et al., 2005). Endophyte containing plants grow faster than the non containing ones (4) Cheplick et al., 1989). Endophytes would have enhanced the hosts' uptake of nutritional elements such as nitrogen and phosphorus (5) Gasoni and Gurfinkel, 1997; (6) Malinowski and Belesky, 1999).

Attempts are being made to isolate and identify bioactive metabolites from endophytic organisms because of its economical effects. E.caducifolia popularly known as a 'dandi thor' or 'Haines' is a cactus, mainly found in all over india. Thus, it is observed that it has a vast range of therapeutic claims and is used in skin disorders; gastro-intestinal diseases, gynaecological and obstetrical problems; respiratory diseases; musculo-skeletal disorders, ENT disorders, immune disorders and as an antidote in snake-bite, scorpion bite. E. caducifolia has also been reported for its wound healing, antimicrobial, antibacterial, antifungal (7) Goyal, Sasmal and Nagori, 2012) and anti nematologiacal activities (8) Maqbool, Hashmi and Ghaffar, (1987). Phylloclade dried biomass of E. caducifolia has potential as a renewable source for biofuel (9) Khan et al., 2013). Laticifers in its phylloclade have also been reported for potential hydrocarbon yielding (10) Rajeswari, et al., 2014). Its juice inhibits leukocyte migration which acts as a catalyst in the development of inflammatory diseases; it may also provide instant relief from Dysmenorrhea (11) Mirabi et al., 2014).

2. MATERIAL AND METHODS

Mature and asymptomatic plant of *Euphorbia Caducifolia* was collected from latitude of 23.9944° N and longitude of 72.4099° E Pasvadal village located in Vadgam Tehsil in Banaskantha district of Gujarat. The sample was collected in sterilized polythene zip lock bag, transported in the lab. Surface sterilization method by (12) Zin et al (2007) used and plant parts were inoculated in respective media latex was spread.

At first all isolates were tested for their phenotypic features. Gram's reaction was performed and the cell shape was observed under light microscope by taking adequate amount of bacterial suspension in sterile physiological solution. Antimicrobial activity against the test pathogens was checked.

2.1 Antimicrobial Activity

Antimicrobial potentials of test cultures were carried out by agar well diffusion method. Antibacterial and Antifungal activity were screened using dual culture method in which both test endophytes and pathogens were inoculated in same media plates. Suspension of pathogenic bacteria and fungi was spread on N. Agar and test cultures were inoculated in wells. By measuring the zone of inhibition antimicrobial activity was calculated. For antibacterial activity *Escherichia coli, Serratia marscesence , Salmonella typhi, Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris, Enterobacter aerogenes, Pseudomonas aeruginosa* were used.

For antifungal activity *Macrofornina phaseolina, Fusarium oxysporium, Sclerotinum rolfsii* were used. On the basis of zone of inhibition three isolates were selected for further process.

2.2 Extraction Of bioactive compounds

Selected bacterial isolates were grown in nutrient broth and for the extraction of bioactive substances ethyl acetate was used as a solvent. 10 ml activated broth was taken in separating funnel, equal amount of ethyl acetate was added and continuous strokes for 5 minutes were given. After the settling of layers lower layer was discarded and process was repeated for three times and extracts were collected in screw cap bottles and kept those in refrigerator (13) Das et al., 2017).

2.3 Preliminary Qualitative tests

Phenols:

- For Ferric chloride test extract was collected, 5 ml distilled water and few drops of 5% ferric chloride solution is added for the development of blue/green colour.
- For lead acetate test 3 ml 10% lead acetate was added in 1 ml extract. White precipitates show the positive test. (14) Banu et al., 2015)

Steroids:

- Libermann Buchard test was performed, 2 ml acetate acid + 2 ml concentrated sulphuric acid added in 1 ml extract, violet to blue/green colour appear.
- Few drops of glacial acetic acid and concentrated hydrochloric acid were added in 1ml extract, violet to blue/green colour developed in Libermann sterol test. Banu et al., 2015)

Flavanoids:

- In 1 ml extract add 1 drop of sodium hydroxide and diluted hydrochloric acid yellow colour of solution was disappear.
- Red precipitates were observed when Zn dust and hydrochloric acid were added in 1 ml extract. Banu et al., 2015)

Tannins

- In 1 ml extract add 1 ml 2% lead acetate solution white precipitates indicate positive test.
- On the addition of potassium dichromate solution in 1 ml extract precipitates appeared. (Banu et al., 2015)

Saponins

- Frothing test; In 1 ml of extract add 5 ml distilled water and shake vigorously forth formation indicates positive test. (Banu et al., 2015)

Alkaloids

- Mayer's test; 1 ml extract and 2 ml Mayer's reagent was added, dull white precipitates show positive test.
- In Dragendroff test reagent was added and orange precipitates indicate positive test.
- Wagner's reagent was added and if reddish brown precipitation observed it show positive test. (15) W.C Evans 2002)

3. RESULTS AND DISCUSSION

Bacterial isolated which were giving best results in terms of antimicrobial activity were selected for further analysis. Names are given accordingly. Three isolates which showing best zones were: As, Ts, Tp.

3.1 Antimicrobial activity

Antimicrobial and antifungal activity was performed to check the efficiency of bacterial isolates. Three bacterial isolates were selected on the basis of zone of inhibition by agar well diffusion method. Zone of inhibition was measured in terms of mm.

Table -1: Antibacterial activity of three bacterial isolates

Isolates	Zone of inhibition(mm)							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Ap	22	30	24	24	27	30	-	28
Ts	19	35	18	35	32	31	-	27
Tp	29	22	37	42	20	25	-	25

Where A=*E.coli*, B=*S.marscescens*, C=*S.typhi*, D=*P.vulgaris*, E=*Ps.aeruginosa*, F=*S.aureus*, G=*E.aerogeneus*, H=*M.leuteus*

Table -2: Antifungal activity of three isolates

Isolates	Zone of inhibition(mm)			
	F_1	F_2	F_3	F_4
Ap	22	19	17	33
Ts	21	26	18	35
Tp	26	29	21	33

Where F1= Macrofornina phaseolina, F2= Fusarium oxysporium(White), F3= Fusarium oxysporium(pink), F4=Sclerotinum rolfsii

Table -3: Results of Qualitative analysis of Bioactive compounds as Phytochemicals

Phytochemical Tests	Result		
	Ap	Ts	Tp
♦ Alkaloids:			
1. Mayer's Test	-	-	-
2. Dragendroff's Test	-	-	-
3. Wagner's Test	++	+ +	+ + +
♦ Saponins:			
1. Frothing Test	+ +	+	+ + +
♦ Tannins:			
1. Lead Acetate Test	+ +	+ + +	+ +
2. Potassium Dichromate Test	-	-	-
♦ Phenols:			
1. Ferric Chloride Test	-	-	-
2. Lead Acetate Test	+ +	+ +	+ +
♦ Steroids:			
1. Libermann Burchard Test	-	-	-
2. Libermann Sterol Test	-	-	-
♦ Flavonoids:			
1. Alkaline Reagent	-	-	-
2. Zn Hydrochloride Reduction Test	-	-	-

Key: (+++) Maximum, (++) moderate, (+) minimum, (-) negative

Further qualitative and Quantitative tests were also performed and for further confirmation TLC was also performed. Qualitative phytochemical screening using chloroform, acetone, ethanol, methanol and aqueous extracts was performed to determine the presence of many phytochemicals like alkaloids, flavanoids, phenols, tannins, saponins, terpenoids.

Endophytic bacteria are attracting increasing attention not only for their promotion of plant growth and control of plant diseases (16) Backman, P. Avet al., (2008), but also for their stress tolerance and improvement of plant growth in an extreme environments (17) Jha *et al.* 2011. Endophytes play a major role in physiological activities of host plants influencing enhancement of stress tolerance, nematode and disease resistance; (18) Hallmann and Siora 1996; (19) Azevedo et al 2000).

4. CONCLUSIONS

From the present study it can be concluded that endophytes are metabolically active within their hosts and play a vital role in maintaining the host endophytic mutualistic balance. From cactus *Euphorbia caducifolia* was collected from Banaskantha district of Gujarat state. Total ten bacterial isolates were recovered. Among them three bacterial isolates namely AP, TS, TP were showing the best antimicrobial activity. They were having capability of producing Biochemical compounds as phytochemicals such as phenol, alkaloids, tannins and saponins. Apart from that it is also having properties of good antimicrobial agent and source of secondary metabolites Phenols, Alkaloids, Tannins, Saponins and this bacterial isolate may act as anticancer, anti diabetic, antiulcerogenic and antiobessicity agents. From plant *Euphorbia caducifolia* isolated endophytic bacteria are showing the 93.43% similarity to *Micrococcus luteus,* 97.97% *Chryseomonas luteola*, and 99.41% *Enterobacter agglomerans* according to Bergey's manual of systematic bacteriology.

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