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ANTIMICROBIAL POTENTIAL OF WILD AND MICROPROPAGATED *Meizotropis Pellita* - AN ENDEMIC AND ENDANGERED PLANT OF KUMAUN HIMALAYAS

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ABSTRACT

Comparative analysis of leaf extracts of micropropagated and wild Meizotropis Pellita was conducted against eight highly pathogenic bacteria. Methanol, Hexane and Aqueous crude extracts from dried leaves of wild and micropropagated plants were used for the study. Standard Agar Well Diffusion and Minimum Inhibitory Concentration (MIC) determining techniques were deployed to determine antimicrobial potential and variation in micropropagated plant in respect to wild specimen. Methanol extract from wild plant presented a prominent Zone of Inhibition (ZOI) and MIC with K.pneumoniae, values were determined to be 28.67±0.67mm and 0.222±0.022/ml respectively. Similarly methanol extract of in vitro raised plant showed ZOI of 26.67±0.88 and MIC of 0.289±0.022 mg/ml with K.pneumoniae, whereas ZOI of 10.33±0.88mm was observed when 30µl of 30µg/ml Gentamycin was used with K.pneumoniae. Which is highly promising, similar results were obtained with other bacteria too. Results indicate that both the specimen contain very potent antimicrobial properties hence could be utilized for pharmaceutical research. When compared with wild almost similar antimicrobial potential was observed in micropropgated plant. Hence it could be concluded that not much difference has accumulated in the micropropagated plant.

Keywords : Antimicrobial, Meizotropis Pellita, Patwa, ZOI, MIC

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I INTRODUCTION

Most of the life forms except plants have evolved highly sophisticated organs by virtue of which they could easily escape from the forces of nature and microorganisms. But plants have lagged behind in such evolutions. Hence they have developed a separate mechanism of synthesizing and storing a wide variety of chemical compounds to counter any circumstance of exposure to such harmful microbe population. These stored compounds since the advent of human race had been harvested and utilized in form of traditional medicine in various parts of world.

The antimicrobial compounds found in plants are of great interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections **[1],[2],[3],[4],[5],[6][7]**. Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) that are under scrutiny as suspected cancer causing agent**[8],[9],[10],[11].**

One such plant species thrives in the extremes of Himalayan region it is *Meizotropis pellita* or Patwa in local dialect. It was first reported by an English botanist Osmonston in 1925 at Patwadanger. It belongs to fabaceae family. Like other legume plants it produces viable seeds. But in wild the plants usually grow from perennial rootstocks in the months of March – April. There are just 200-300 specimens left at a distance of 12 km from district headquarters of Nainital, Uttarakhand, one of the most famous hill stations of India. Records speak that Patwa existed in the Kali Kumaun and Dhoti district of Nepal as well. However, the species could no longer be traced in that region of Nepal now. This endangered and endemic plant could be a rich source of some of very potent antimicrobial compounds but its capabilities have not been tested and presented to the world due to its scarce availability.

Aim of our study is to determine presence of such antimicrobial compounds in Patwa in order to increase its economic value and hence conservation efforts. We also aim to study changes if any accumulated in *in vitro* raised plant due to micropropagation technique.

II MATERIAL AND METHODS

Leaves of the wild plant were collected from Patwadanger, 12 Km from district headquarters of Nainital. And leaves of the *in vitro* raised hardened plant were collected from the Greenhouse of Department of Biotechnology, Kumaun University Nainital, Bhimtal Campus, Bhimtal, Uttarakhand in the month of June 2015 with average temperature ranging in between 14-26 degree Celsius.

The leaves after collection were soaked with 1% Tween 20 solution for 5 minutes and then were rinsed with analytical grade water. Following it they were placed on blotting paper and kept for drying 48 hours under room temperature following which they were moved to dry air oven at 50ºC for another 48 hours of drying. And finally

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the dry leaves were ground into fine powder. Finely ground leaf powder was weighted and added 10 g/flask to three pairs of conical flasks containing 100ml of Hexane, Methanol and analytical grade double distilled water respectively making it 10g/100ml or a 1:10 solute to solvent ratio. The flasks were tightly closed with cotton plugs and placed in rotatory shaker at 25ºC and 120 RPM for 48 hours completing which the solvent was filtered out using Whatman filter paper No-1 and stored under refrigerator at 4ºC. Fresh solvent was added to flask again and the process was repeated one more time for a better extract yield. Once entire filtrate was collected it was subjected to evaporation in water bath shaker at temperatures not exceeding 50ºC till a dry mass was obtained. The extracts were stored in refrigerated conditions till use.

All the microorganisms used in the study were procured from Institute of microbial Technology, Chandigarh; *Kliebsella pneumoniae* (MTCC 3384), *Proteus vulgaris* (MTCC 1771), *Bacillus subtilis* (MTCC 441), *Salmonella typhimurium* (MTCC 3224), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), *Aeromonas salmonicida* (MTCC 1522) and *Aeromonas hydrophila* (MTCC 646). Fresh overnight grown cultures of microbes were prepared in nutrient broth for inoculation from the master culture.

2.1 Antimicrobial activity through Agar Well Diffusion

The antibacterial activity was determined using Agar well diffusion method **[12]**. Sterile Nutrient agar plates were prepared and gel borer was utilized to cut wells in the gel. Each well hence produced was poured with 30µl of 100mg/ml distinct extract re-suspended in 5% DMSO. Negative control for each microbial culture was established by pouring well with 30µl of 5% DMSO absent any extract. The plates were kept for 1 hour under room temperature inside laminar for extracts to diffuse properly. Then the microbial culture 90µl per plate was inoculated and spread properly using a spreader. The plates hence obtained were kept overnight at 37ºC in a bacteriostatic incubator. Triplicates were performed for each plate to overcome experimental errors. Similarly 30µl of 30µg/ml of Ampicillin sodium salt, and Gentamycin sulphate prepared in 5% DMSO were also poured in wells for all the eight microorganisms to obtain a standard for comparison of effectively of extracts against selected microbes.

2.2 Minimum Inhibitory Concentration (MIC)

Slightly modified method **[13]** was used to prepare 1ml of each extract (1, 2, 3, 4, 5, 10, 15, 20 mg/ml) prepared in 5% DMSO was added to test tubes containing freshly prepared 14ml Nutrient broth; following which 10µl bacterial culture was inoculated and incubated of 24 hours at 37ºC the test tubes were analysed for microbial growth both via unaided eye and UV-VIS spectrophotometry at 620nm wavelength. Controls were prepared by adding 1ml of respective concentration extracts in 14ml of Nutrient Broth but no bacteria was inoculated into them. If MIC was not obtained at 15mg/ml concentration it was assumed that MIC ≥20mg/ml and was further not investigated.

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III RESULTS AND DISCUSSION

As clearly indicated by results in following Table 1 and Table 2 crude extract from both the plants possesses a very strong antimicrobial activity against all of highly pathogenic microbial forms, not much difference was observed in wild and micropropagated plant. Results are indicative to the possible fact of the presence of novel combination of compounds which possesses strong activity against microbes. Presence of such a strong antimicrobial activity could be a result of its natural habitat. The plant grows on high altitude in the canopy of Chir forests of Patwadangar amidst many other varieties of shrubs and herbs. Due to the dense vegetation and shedding of leaves by tall trees the floor of the forest is constantly overburdened with huge quantities of dead and decaying organic waste. These situations are ideal for growth of a variety of microbes. Hence for a plant to sustain life and continue proliferation in such harsh conditions it needs to commission artillery of compounds to fight against multiple kinds of predatory infections. This could possibly explain the presence of such an effective activity against wide range of highly pathogenic bacteria.

IV FIGURES AND TABLES

Table 1 – Antimicrobial activity of *Meizotropis pellita* leaves through agar well diffusion

Zone of inhibition (mm)								
Microorganism	**Wild plant (100mg/ml)**			***In vitro* raised Plant (100mg/ml)**			**Standard Antibiotic (30µg/ml)**	
	Methanol	**Hexane**	**Aqueous**	**Methanol**	**Hexane**	**Aqueous**	**Gentamycin**	**Ampicillin**
B. subtilis	**10.00±0.58^{c}**	0.00±0.00^{a}	0.00±0.00^{a}	9.33±0.33^{c}	0.00±0.00^{a}	0.00±0.00^{a}	17.33±0.333^{e}	1.33±1.333^{a}
A. salmonid	13.67±0.33d^{e}	0.00±0.00^{a}	0.00±0.00^{a}	**15.67±0.33^{d}**	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.000^{a}	0.00±0.000^{a}
P. vulgaris	14.67±0.88^{e}	13.33±0.33^{d}	0.00±0.00^{a}	**15.33±0.67^{d}**	11.00±0.58^{e}	0.00±0.00^{a}	15.33±0.333^{d}	0.00±0.000^{a}
S.typhimurium	3.00±1.53^{b}	**10.00±.058^{c}**	0.00±0.00^{a}	7.67±0.67^{b}	8.67±0.33^{d}	0.00±0.00^{a}	14.67±0.667^{cd}	7.00±.577^{b}
A.hydrophila	14.00±0.58^{e}	6.33±0.33^{b}	0.00±0.00^{a}	**14.33±0.67^{d}**	7.67±0.33^{d}	0.00±0.00^{a}	17.00±0.577^{e}	21.67±.882^{c}
E. coli	**11.67±0.33c^{d}**	6.00±0.00^{b}	0.00±0.00^{a}	10.33±0.33^{c}	6.67±0.33^{c}	0.00±0.00^{a}	13.67±0.333^{c}	0.00±0.000^{a}
P.aeruginosa	10.00±0.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}	**14.00±0.58^{d}**	0.00±0.00^{a}	0.00±0.00^{a}	16.00±0.577^{de}	0.00±0.000^{a}
K.pneumoniae	**28.67±0.67^{f}**	6.00±0.58^{b}	11.00±0.58^{b}	26.67±0.88^{e}	5.67±0.33^{b}	9.67±0.88^{b}	10.33±0.882^{db}	0.00±0.000^{a}

Note - Values represent mean± standard error of Zone of Inhibition, significant at p= 0.05 level (Duncan's multiple range test); Values superscripted by same letter in a column are not significantly different; The experiments were performed in triplicates. Values highlighted bold represent best activity against a specific microbe.

Table 2– Minimum inhibitory Concentration of *Meizotropis pellita* leaf extracts

Minimum inhibitory Concentration (mg/ml)						
Microorganisms	**Wild plant**			***In vitro* raised Plant**		
	Methanol	**Hexane**	**Aqueous**	**Methanol**	**Hexane**	**Aqueous**
B. subtilis	**0.267±0.038** [a]	1.111±0.111 [de]	1.111±0.111 [bc]	0.289±0.022 [a]	0.889±0.111 [bc]	1.222±0.111 [bc]
A. salmonid	**0.333±0.000** [a]	0.889±0.111 [bcd]	1.000±0.000 [b]	0.378±0.045 [ab]	1.111±0.111 [c]	0.889±0.111 [ab]
P. vulgaris	**0.311±0.022** [a]	0.422±0.045 [a]	1.222±0.111 [bc]	0.378±0.045 [ab]	0.467±0.000 [a]	1.333±0.000 [c]
S.typhimurium	1.111±0.111 [c]	**0.778±0.111** [bc]	1.333±0.000 [c]	0.889±0.111 [c]	0.889±0.111 [bc]	1.222±0.111 [bc]
A.hydrophila	**0.378±0.045** [a]	1.000±0.000 [cde]	1.333±0.000 [c]	0.422±0.045 [ab]	0.889±0.111 [bc]	1.222±0.111 [bc]
E. coli	**0.600±0.067** [b]	0.778±0.111 [bc]	1.111±0.111 [bc]	0.534±0.067 [b]	0.667±0.000 [ab]	0.889±0.111 [ab]
P.aeruginosa	**0.667±0.000** [b]	1.222±0.111 [e]	1.222±0.111 [bc]	0.778±0.111 [c]	1.111±0.111 [c]	1.333±0.000 [c]
K.pneumoniae	**0.222±0.022** [a]	0.600±0.067 [ab]	0.6670.000± [a]	0.289±0.022 [a]	0.711±0.155 [ab]	0.711±0.155 [a]

Note - Values represent mean± standard error of Minimum Inhibitory Concentration, significant at p= 0.05 level (Duncan's multiple range test); Values superscripted by same letter in a column are not significantly different; The experiments were performed in triplicates. The experiments were performed in triplicates. Values highlighted bold represent best activity against a specific microbe.

V CONCLUSION

It is well established that the plants store a wide range of compounds which possess antimicrobial property. But something is very important and peculiar about *Meizotropis pellita*. It could be inferred from the result of experiment that Patwa comprises of highly robust antimicrobial compounds which have a broad spectrum of activity against tested pathogens. Methanol extract specifically presented a very strong antimicrobial activity against all the test microbial strains. For some microbes it even surpasses the activity presented by the standard antibiotics, which clearly indicates the plant is a source to some very unique and powerful antimicrobial compounds which needs further advanced investigation. This clearly should make this plant a blue eyed boy for the pharmaceutical companies to research and invest on. This should also help preventing this rare plant possessing such economic value from extinction.

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