

Antioxidant And Polyphenol Activity of Alkaliphilic Isolates

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ABSTRACT

Extremophiles can produce antioxidants and polyphenols in order to keep the cell protected from oxidants and extreme conditions. In order to test this hypothesis the samples were taken from the alkaline sites. Various bacterial isolates were isolated. These organisms were grown in the alkaline broth medium. Study of antioxidant and polyphenol activity was performed from the cell free extracts. The results were not consistent hence method was modified. Cell free extracts were dried for few days and then antioxidant and polyphenol activity of the extracts were detected. But these cell free extracts didn't show antioxidant and polyphenol activity. It is also possible that the bacteria can accumulate these antioxidants inside the cell. Hence intracellular extracts of the bacteria were prepared by lysing the cells with the glass beads and extraction with methanol. These intracellular extracts were dried at room temperature. The antioxidant and polyphenol activities were detected.

1. Introduction

Today antioxidants become essential for life. Antioxidants can slow the aging process. Extremophiles can produce wide array of compounds for their survival. They can also produce the compounds which protect them from UV light and other drastic conditions. Hence exploration of extremophiles can contribute for solving the problems of humanity. In today's world pollution has increased. This can decrease the immunity and increase the aging process of the individuals. Microorganisms such as bacteria, fungi and actinomycetes have potential to produce novel bioactive metabolites (1). Antioxidants have ability to scavenge the free radicals. Antioxidants deals with oxidative stress and helps to reduce free radicals formed during the biochemical processes. Hunting of natural oxidants becomes challenge for cosmetic and pharmaceutical industries due to which research on the antioxidants have increased (2). The antioxidants which work at broad pH range may find several applications. Hence antioxidants from extremophiles can have potential to tolerate drastic environmental conditions.

2. Material and methods

Alkaliphilic isolates were inoculated into tryptic soy broth (pH-11) and incubated for 10-14 days at 30°C. Cell free extracts were prepared by centrifugation at 13500 rpm for 30 minutes. Cell free extracts were used for the detection of antioxidant and polyphenol activity. These cell free extracts were dried at room temperature for 10-15 days. After drying antioxidant and polyphenol activity were detected. For extraction of intracellular metabolites, cell pellets were used. The cell pellet was then extracted using saline and methanol. The cells were lysed by using glass beads in order to extract intracellular or membrane bound metabolites. These extracts

were then kept at refrigerator for cold extraction. These intracellular extracts then dried at room temperature for the period of 10-15 days. After drying these intracellular extracts were used for detection of antioxidant and polyphenol activity. Antioxidant activity was detected by using 0.2mM DPPH solution. Vitamin C was used as standard. Known amount of extracts were taken and volumes were adjusted with distilled water. DPPH solution was added in cell free and intracellular extracts followed by incubation at room temperature for 40 minutes in dark. Absorbance was recorded at 515nm using colorimeter. (1), (3) and (4). Polyphenol activity was detected by using gallic acid as a standard and using Folin Ciocalteu reagent along with 20% sodium carbonate solution followed by incubation at room temperature 40 minutes in dark. Absorbance was recorded at 650nm (6). Total phenolic content was detected by calibration curve of the standard. Results were expressed as mg of gallic equivalent per ml of the sample.

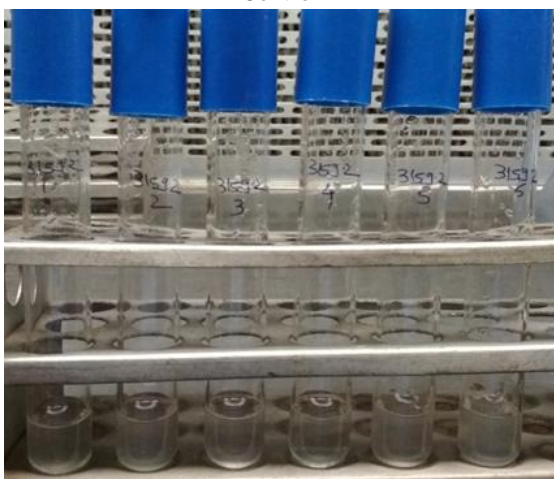
3. Results and Discussion

Antioxidant activity of extremophilic alkaliphiles 1-6
Table-1

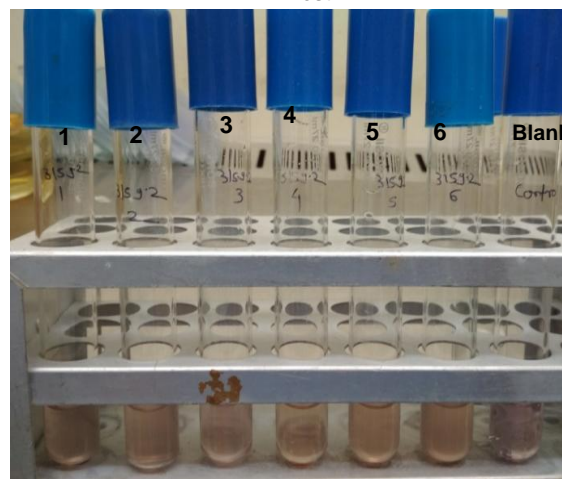
| Intracellular dried extract no. | Activity in percentage % per 200µl |
|---------------------------------|------------------------------------|
| 1 | 50 |
| 2 | 56.25 |
| 3 | 43.75 |
| 4 | 62.5 |
| 5 | 43.75 |
| 6 | 12.5 |
| Blank | |

Antioxidant activity of extremophilic alkaliphiles 1-6

Photograph no.1
Control



Photograph no.2
Test



Graph-1

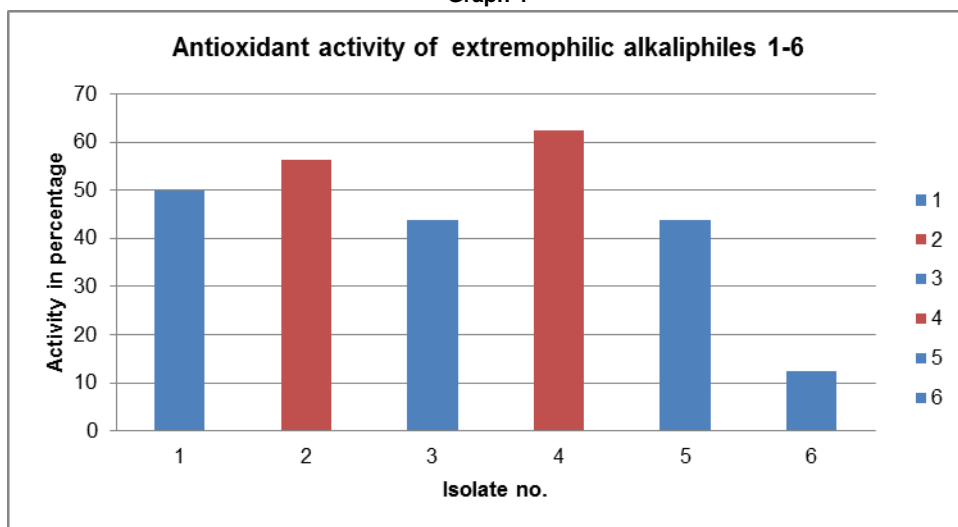


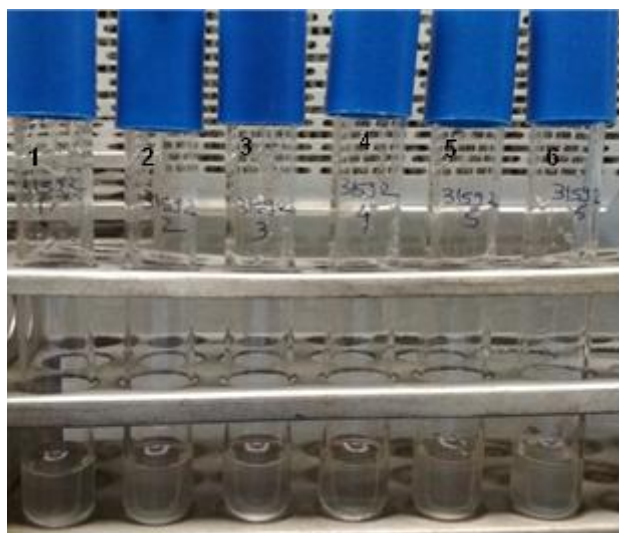
Table-2

Polyphenol activity of extremophilic alkaliphiles 1-6

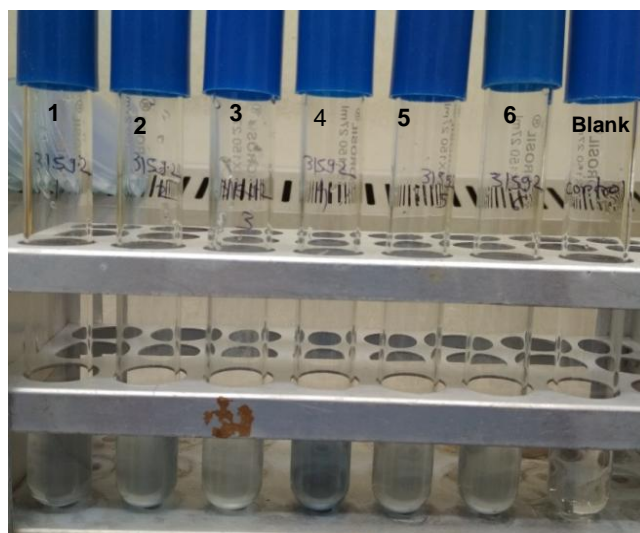
| Dried intracellular extract | mg of GAE (Gallic Acid Equivalent) per ml |
|-----------------------------|--|
| 1 | 36.36 |
| 2 | 27.27 |
| 3 | 21.21 |
| 4 | 78.78 |
| 5 | 24.24 |
| 6 | 18.18 |

Polyphenol activity of extremophilic alkaliphiles 1-6

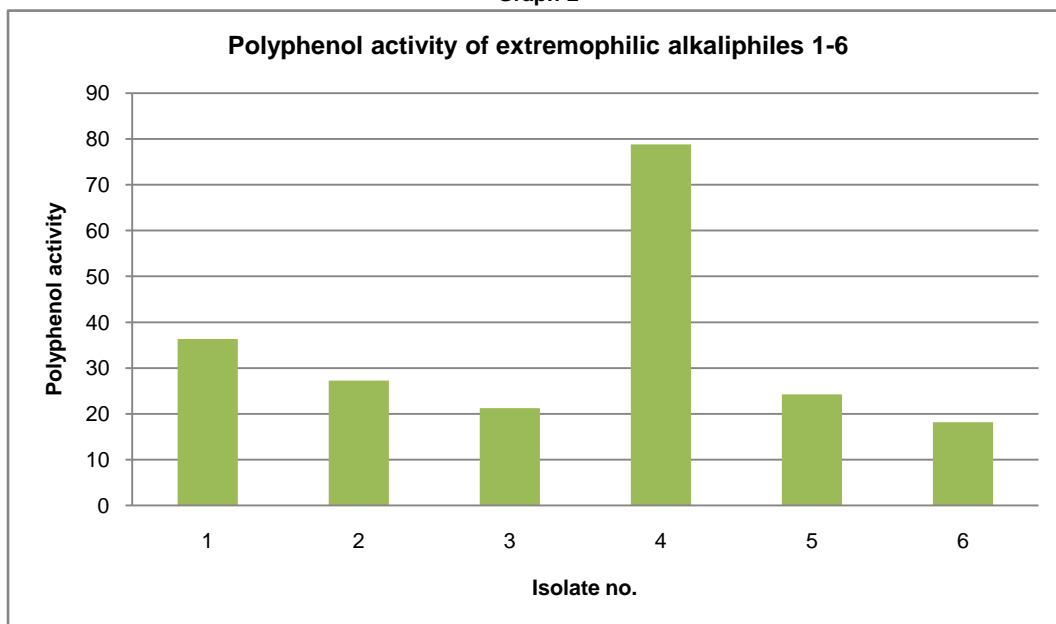
Photograph no.3
Control



Photograph no.4
Test



Graph-2



The alkaliphilic isolate-4 was showed maximum antioxidant and polyphenol activity as compared to other alkaliphilic isolates. Priyanka Chandra and Daljit Singh Arora (1) studied antioxidant activity of fungal isolates of *Aspergillus* by various procedures including DPPH method. They found that *Aspergillus terreus* demonstrated potent antioxidant activity. Ronald L. Prior et al (3) reviewed methods available for measurement of antioxidant activity. Their overview provided a basis and rationale for developing standardized antioxidant capacity methods for the food, nutraceutical and dietary supplement industry. They also demonstrated chemistry behind the various assays available for detection of antioxidant activity. Sascha C.T. and Nicklish et al (5) demonstrated and

optimized the DPPH assay protocol for measurement of antioxidant activity of proteins.

4. Conclusion

Positive correlation between antioxidant and polyphenol activity was observed. These isolates produced antioxidants at alkaline pH. It was also found that these alkaliphiles accumulated these antioxidants inside the cell. Antioxidants and polyphenols produced by these isolates were found stable at alkaline pH. The metabolites of extremophiles can have potent applications in food and cosmetic industries.

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