

***In vitro* microbial production of citric acid by *Aspergillus niger* NCIM-1270 exposed to 6-fluoro-4-hydroxycoumarin**

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Abstract: The current world production of citric acid is estimated to be 1.6 million tonnes per year whereof 99% is produced by microbial fermentation. The impact of 6-fluoro-4-hydroxycoumarin on microbial bioproduction of citric acid by *Aspergillus oryzae* NCIM -949, *Aspergillus fumigatus* NCIM -902, *Aspergillus awamori* NCIM -861, *Aspergillus candidus* NCIM -883 and *Aspergillus niger* NCIM -1270 has been assessed. It has been observed that the fungal strain *Aspergillus niger* NCIM -1270 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., 6-fluoro-4-hydroxycoumarin at molar concentration, i.e., $3.0 \times 10^{-5} \text{M}$ has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM -1270 and enhances the yield of citric acid to an extent of 4.915% higher in comparison to control fermentor flasks, i.e., 3.006 g/100 mL under the optimized conditions, viz. 29°C temperature, 2.1 pH, 12 days of incubation period with 24% (w/v) molasses solution.

(Key words : Molasses, citric acid fermentation, 6-fluoro-4-hydroxycoumarin and *Aspergillus niger* NCIM -1270)

Introduction

Coumarins are reported to involved in the process of growth regulatha and protecting against viral disorders in figs. Coumarins show characteristic pharmacological properties such as anti bacteriostatic antimytotic and antibacteruistatuc antimytotic and anti HIV activity.

Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of coumarins have been identified from natural sources, especially green plants. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Among these properties, their cytotoxic effects were most extensively examined. In this review, their broad range of effects on the tumors as shown by various *in vitro* and *in vivo* experiments and clinical studies are discussed. Hence, these cytotoxic coumarins represent an exploitable source of new anticancer agents, which might also help addressing side-toxicity and resistance phenomena. These natural compounds have served as valuable leads for further design and synthesis of more active analogues. In this review, plant derived coumarins and their synthetic analogues were systematically evaluated based on their plant origin, structureactivity relationship and anticancer efficacy. Owing the their diverse effects and inconclusive results from different *in vitro* studies, the mechanism of their action is not yet fully understood and correlation of effects with chemical structures is not conclusive at the moment. It is the objective of this review to summarize experimental data for different coumarins, used as cytotoxic agents, because promising data have been reported for a series of these agents. Yet, the results from different

Table - 4
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Concentration of coumarin used A x 10 ^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% difference of citric acid increased after 8,12 and 16 days
Control	8	5.9066241	5.6323633	—
(-) Coumarin	12	6.9934239	4.7320658	—
	16	6.4905311	4.6365869	—
1.0 x 10 ⁻⁵ M	8	5.9195373	5.6273950	+ 0.2186223
(+) Coumarin	12	7.0364845	4.7289056	+ 0.6157298
	16	6.5175932	4.6280709	+ 0.4169473
2.0 x 10 ⁻⁵ M	8	5.9598937	5.618345	+ 0.9018620
(+) Coumarin	12	7.1273989	4.7194256	+ 1.9157282
	16	6.5889891	4.6198059	+ 1.5169482
3.0 x 10 ⁻⁵ M**	8	6.0672029	5.6077360	+ 2.7186223
(+) Coumarin	12	7.3372016***	4.7088641	+ 4.9157280
	16	6.6993280	4.6094253	+ 3.2169462
4.0 x 10 ⁻⁵ M	8	6.0376698	5.6284103	+ 2.2186226
(+) Coumarin	12	7.2882477	4.7264340	+ 4.2157290
	16	6.6733659	4.6291856	+ 2.8169466
5.0 x 10 ⁻⁵ M	8	5.9786035	5.6399867	+ 1.2186216
(+) Coumarin	12	7.2392937	4.7398206	+ 3.5157285
	16	6.6399233	4.6480482	+2.3016945
6.0 x 10 ⁻⁵ M	8	6.0081367	5.657613	+1.7186229
(+) Coumarin	12	7.2113200	4.7581603	+3.1157284
	16	6.6214517	4.6556830	+2.0170140
7.0 x 10 ⁻⁵ M	8	5.9845102	5.6791815	+ 1.3186229
(+) Coumarin	12	7.1893497	4.7781403	+ 2.8015719
	16	6.6019701	4.6788203	+ 1.7169473
8.0 x 10 ⁻⁵ M	8	****	—	—
(+) Coumarin	12	****	—	—
	16	****	—	—
9.0 x 10 ⁻⁵ M	8	****	—	—
(+) Coumarin	12	****	—	—
	16	****	—	—
10.0 x 10 ⁻⁵ M	8	****	—	—
(+) Coumarin	12	****	—	—
	16	****	—	—

* Mean of three observations ** Optimum concentration of coumarin

*** Optimum yield of citric acid (+)ve values indicate % increase in the yield of citric acid.

Experimental deviation (±) 1.5% to 3.5% **** Insignificant valid.

coumarins with various tumor lines are contradictory in part. We therefore conclude that there is still a long way to go until we know which cytotoxic agent will clinically be suitable for what tumor entity for treatment. Their ability to bind metal ions represents an additional means of modulating their pharmacological responses.¹⁻⁵

Coumarins in the field of biotechnology has assumed great importance. Some coumarins and its derivative are also used in medicine today, and many attempts have been to established the structure- activity relationship of some coumarin derivatives. The correlation of chemical structure with anticoagulant activity of some coumarin derivatives has also been studied by many workers⁶⁻¹⁵.

Literature survey reveals that little work has been done on efficacy of coumarins on citric acid fermentation¹⁶⁻²⁴. Therefore, the author has employed 6-fluoro-4-hydroxycoumarin on citric acid fermentation by *Aspergillus niger* NCIM - 1270

Experimental

The influence of 6-fluoro-4-hydroxycoumarin on facile transformation of molasses to citric acid by *Aspergillus niger* NCIM - 1270

The composition of the production medium on citric acid fermentation by *Aspergillus niger* NCIM-1270 has been prepared as follows : Molasses : 24% w/v, NH_4NO_3 : 1.2 % KH_2PO_4 : 1.2 %, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$:1.2 %, pH: 2.1

The pH of the production medium was adjusted to 2.1 by adding requisite amount of KCl-HCl buffer solution and this pH was also ascertained by a pH meter.

The above composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for citric acid fermentation by *Aspergillus niger* NCIM-1270.

Now, the same production medium for

citric acid fermentation by *Aspergillus niger* NCIM-1270 was prepared for 99-fermentor flask, i. e; each contained '100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermentor flasks. The remaining 9-fermentor flasks out of 99-fermentor flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermentor flasks.

After preparing the above sets of fermentor flasks M/100 solution of 6-fluoro-4-hydroxycoumarin was prepared and from the above coumarin solution; 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no 6-fluoro-4-hydroxycoumarin.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 6-fluoro-4-hydroxycoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below:
 $A \times 10^{-x}$ M i.e., 1.0×10^{-5} M to 10.0×10^{-5} M respectively

Where : A=amount of coumarin in ml, i.e., 1.0 ml .. to 10 ml. x= Molarity of the coumarin solution respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 291°C and analysed colorimetrically after 8,12 and 16 days for citric acid²⁵ formed and molasses²⁶ left unfermented.

Results and Discussion

The results of the influence of the coumarin, i.e., 6-fluoro-4-hydroxycoumarin on *in vitro* production of citric acid by *Aspergillus niger* NCIM-1270 has been recorded in the table-

1. It is obvious from the results that the presence of above coumarin in the production medium has enhanced the yield of citric acid at its all molar concentration but to a lesser amount.

It has been noticed that the presence of above coumarin compound, i.e., 6-fluoro-4-hydroxycoumarin was found to be beneficial and encouraging for the higher yield of citric acid up to its concentration from 1.0×10^{-5} M to 3.0×10^{-5} M but not so significant and above this concentration the coumarin under trial has inhibitory effect and retarded the yield of citric acid. However, it is interesting to note that *in*

vitro production of citric acid by *Aspergillus niger* NCIM-1270 exposed to experimental concentration, i.e., from 1.0×10^{-5} M to 7.0×10^{-5} M of coumarin under trial has been found to be slight better than the control fermentor flasks.

The maximum citric acid production was observed at 3.0×10^{-5} M concentration of 6-fluoro-4-hydroxycoumarin on *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270, i.e., 7.3372016g/100ml which is 4.9157280% higher in comparison to control fermentor flasks, i.e., 6.9934239 g/100 ml in 12 days of optimum incubation period.

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