

## ***In vitro* microbial production of citric acid by *Aspergillus niger* NCIM-1270 exposed to 3-acetylcoumarin**

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**Abstract :** The efficacy of 3-acetylcoumarin on *in vitro* microbial transformation of molasses to citric acid by *Aspergillus niger* NCIM-1270 has been assessed. It has been found that the coumarin, i.e, 3-acetylcoumarin under trial has stimulatory effect on *in vitro* microbial transformation of molasses to citric acid by *Aspergillus niger* NCIM-1270 and enhances the yield of citric acid to an extent of 8.715 % higher in comparison to control fermentor flasks, i.e., 6.955 g/100mL in 12 days of incubation period 2.1 pH and 29°C temperature with 24% vol. molasses solution.

**(Key words :** Molasses, citric acid fermentation, 3-acetylcoumarin, and *Aspergillus niger* NCIM-1270)

### **Introduction**

Coumarins are an important class of compounds of both natural and synthetic origin. Many compounds that contain the coumarin moiety exhibit useful and diverse pharmaceutical and biological activities, and there has been a growing interest in their synthesis. Some of these coumarin derivatives have been found useful in photochemotherapy, antitumor and anti-HIV therapy, as CNS-stimulants, antibacterial, anticoagulant, antifungal, and antioxidant agents, and as dyes. Natural, semi-synthetic and synthetic coumarins are useful substances in drug research. Coumarins can be used not only to treat cancer, but also to treat the side effects caused by radiotherapy.

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 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.

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 establish 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 workers<sup>1-32</sup>. Literature survey reveals that no work has been done on efficacy of coumarins on citric acid fermentation. Therefore, the author has employed 3-acetylcoumarin on citric acid fermentation by *Aspergillus niger* NCIM-1270

## Experimental

The influence of 3-acetylcoumarin on *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270. The composition of the production medium for *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270 has been prepared as follows :

Molasses : 24% (w/v),  $\text{NH}_4\text{NO}_3$  : 1.2%  
 $\text{KH}_2\text{PO}_4$  : 1.2%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  : 1.2% , pH : 2.1

The pH of the production medium for *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270 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 *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270.

Now, the same production medium for *in vitro* production of citric acid 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/1000 solution of 3-acetylcoumarin 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 coumarin.

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 3-acetylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x} \text{ M}$

$1.0 \times 10^{-5} \text{ M}$  to  $10.0 \times 10^{-5} \text{ M}$

Where : = amount of coumarin, in ml,

i.e. 1.0 ml ..... to 10 ml.

x = Molarity of the coumarin solution

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 29°C and analysed after 8, 12 and 16 days for citric acid<sup>33</sup> formed and molasses<sup>34</sup> left unfermented .

## Results and Discussion

The data recorded in the table-1 shows that the coumain 3-acetylcoumarin was found to be significant for higher production of citric acid up to its concentration from  $1.0 \times 10^{-5}$  to  $6.0 \times 10^{-5} \text{ M}$  and above this concentration the *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270 was found to be decreasing. However, *in vitro* production of citric acid by *Aspergillus niger* NCIM 1270 were higher than that of control fermentor flasks.

The maximum yield of citric acid was observed at  $6.0 \times 10^{-5} \text{ M}$  concentration of the coumarin 3-acetylcoumarin i.e., 7.5612386 g/100

**Table - 1**  
***In vitro* microbial production of citric acid by**  
***Aspergillus niger* NCIM-1270 exposed to 3-acetylcoumarin**

| Concentration of coumarin used<br>A x 10 <sup>-x</sup> M | Incubation Period in days | Yield of citric acid* in g/100 ml | Molasses* left<br>Unfermented in g/100 ml | % difference of citric acid increased after 8, 12 and 16 days |
|----------------------------------------------------------|---------------------------|-----------------------------------|-------------------------------------------|---------------------------------------------------------------|
| Control                                                  | 8                         | 5.8573954                         | 5.6573936                                 | —                                                             |
| (-) Coumarin                                             | 12                        | 6.9550493                         | 4.7730669                                 | —                                                             |
|                                                          | 16                        | 6.4609827                         | 4.6561183                                 | —                                                             |
| 1.0 x 10 <sup>-5</sup> M                                 | 8                         | 5.8819249                         | 5.6486211                                 | (+)0.4187782                                                  |
| (+) Coumarin                                             | 12                        | 7.0117896                         | 4.7539016                                 | (+)0.8158144                                                  |
|                                                          | 16                        | 6.4998586                         | 4.6383212                                 | (+)0.6017025                                                  |
| 2.0 x 10 <sup>-5</sup> M                                 | 8                         | 5.8994971                         | 5.6373683                                 | (+)0.71877852                                                 |
| (+) Coumarin                                             | 12                        | 7.0396098                         | 4.7481603                                 | (+) 1.21581453                                                |
|                                                          | 16                        | 6.5192415                         | 4.6232379                                 | (+)0.9017018                                                  |
| 3.0 x 10 <sup>-5</sup> M                                 | 8                         | 5.9287841                         | 5.6281418                                 | (+) 1.2187789                                                 |
| (+) Coumarin                                             | 12                        | 7.1299355                         | 4.7376983                                 | (+) 2.5145213                                                 |
|                                                          | 16                        | 6.5783803                         | 4.6146395                                 | (+) 1.8170239                                                 |
| 4.0 x 10 <sup>-5</sup> M                                 | 8                         | 5.9932154                         | 5.6194293                                 | (+) 2.3187780                                                 |
| (+) Coumarin                                             | 12                        | 7.2889926                         | 4.7281683                                 | (+) 4.8014512                                                 |
|                                                          | 16                        | 6.6938792                         | 4.6081684                                 | (+) 3.6046606                                                 |
| 5.0 x 10 <sup>-5</sup> M                                 | 8                         | 6.0998762                         | 5.6062493                                 | (+) 4.1397376                                                 |
| (+) Coumarin                                             | 12                        | 7.3943174                         | 4.7032194                                 | (+) 6.3158157                                                 |
|                                                          | 16                        | 6.7979638                         | 4.5975471                                 | (+) 5.2156322                                                 |
| 6.0 x 10 <sup>-5</sup> M**                               | 8                         | 6.1689374                         | 5.5973611                                 | (+) 5.3187804                                                 |
| (+) Coumarin                                             | 12                        | 7.5612386***                      | 4.6948163                                 | (+) 8.7158160                                                 |
|                                                          | 16                        | 6.9078905                         | 4.5818930                                 | (+) 6.9170251                                                 |
| 7.0 x 10 <sup>-5</sup> M                                 | 8                         | 6.0869338                         | 5.6132429                                 | (+) 3.9187793                                                 |
| (+) Coumarin                                             | 12                        | 7.4012724                         | 4.7068263                                 | (+) 6.4158150                                                 |
|                                                          | 16                        | 6.8045147                         | 4.5930261                                 | (+) 5.3170239                                                 |
| 8.0 x 10 <sup>-5</sup> M                                 | 8                         | 5.9815017                         | 5.6231123                                 | (+) 2.1187966                                                 |
| (+) Coumarin                                             | 12                        | 7.2759915                         | 4.7151625                                 | (+) 4.6145208                                                 |
|                                                          | 16                        | 6.6882270                         | 4.6081829                                 | (+) 3.5171785                                                 |
| 9.0 x 10 <sup>-5</sup> M                                 | 8                         | ****                              | —                                         | —                                                             |
| (+) Coumarin                                             | 12                        | ****                              | —                                         | —                                                             |
|                                                          | 16                        | ****                              | —                                         | —                                                             |
| 10.0 x 10 <sup>-5</sup> M                                | 8                         | ****                              | —                                         | —                                                             |
| (+) Coumarin                                             | 12                        | ****                              | —                                         | —                                                             |
|                                                          | 16                        | ****                              | —                                         | —                                                             |

\* Mean of three observations \*\* Optimum concentration of coumarin

\*\*\* Optimum yield of citric acid \*\*\*\* Insignificant value (+)ve values indicate % increase in the yield of citric acid, Experimental deviation (±) 1.5% to 3.5%

ml in 12 days of optimum incubation period which is 8.7158160% higher in comparison to control, ie.,

6.9550493g/100 ml in the same experimental conditions and optimum incubation period.

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