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INHIBITION KINETICS OF *E. COLI* BY METHANOLIC ROOT EXTRACT OF *ALSTONIA SCHOLARIS*

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ABSTRACT

The potential of methanol extract as a potent antimicrobial agent has been observed to be an effective means to combat disease causing pathogenic organisms. Growth pattern of *Escherichia coli* cells showed that the growth of cells decreased with an increase in concentration of extract. The specific relationship between the specific growth rate and the substrate concentration was thus be obtained by fitting these data into Monod kinetic model. The non competitive and uncompetitive inhibition kinetic models were also tested in order to determine the inhibitory effects of the methanol extract of roots on *Escherichia coli.* Longest lag phase of 8 hrs was obtained at nutrient broth concentration of 2 g/L. Kinetic studies showed that with an increase in concentration of extract (10-30 g/L) the specific growth rate and inhibition constant decreased but saturation constant increased. Therefore the biokinetic constant estimated by these models have laid the possibility of obtaining suitable antibiotic from *Alstonia scholaris*.

INTRODUCTION: Whole plants or their parts have been used extensively since time immemorial in treating various disorders and have been alternative to the modern chemical based drugs because of potent side effects. The family Apocynaceae to which *Alstonia scholaris* belongs have more than 250 genera and 2000 different species of tropical plants like *Rauwolfia serpentine*, *Alstonia scholaris, Alstonia venenata* and *Vinca rosea*[1]. Most of these plants have been used against various diseases of skin, heart diseases, liver diseases, dysentery tumors and hypertension.

Alstonia scholaris is a tropical evergreen tree most commonly found in south Asia. So far almost all parts of plants have found to show some therapeutic activity. Leaves, stem bark, roots and inflorescence have shown antimicrobial properties against most common disease causing organisms [1, 2]. The various alkaloids (Echitamine, Ditamine) found in bark have been used in the treatment of diarrohea and malaria [3, 4, 5]. The bark extract is also a useful remedy for treating asthma, lung cancer, hypertension, and pneumonia while the leaf extract is used to treat fever[6]. It is effective against boils and ulcers and can be cured by applying milky latex or young leave as poultice over affected area.

The latex obtained from the various plant parts is useful in treating ailments like ulcers, sores, tumours, and rheumatoid pain [7]. Roots however have been reported to have very rare medicinal use in enlarged liver and pain but no therapeutic intervention is available so far.

The existing data on the antimicrobial activities can be evidenced by the pathogen-extract interaction. It is well recognized by cell biologists that loss of cell viability may be greatly attributed to the large increase in membrane permeability because of the destruction of plasma membrane in the presence of high concentrations of extracts [8]. Therefore the present

study was undertaken to establish the effect of inhibitory substance like methanolic root extract on pathogen using Monod kinetic and the toxic effects of the inhibitor concentration (Methanolic concentration) could be studied using inhibition models viz. Non Competitive and Un Competitive [9, 10, 11].

MATERIAL AND METHODS

Collection and processing of plant materials: The fresh plant parts of *Alstonia scholaris* were collected and powdered which was then subjected to solvent extraction using methanol as a solvent in a soxhlet apparatus. The phytochemical investigation and antimicrobial screening of the various plant extracts were done. The plant extract with highest antimicrobial activity was chosen to proceed with the kinetic modelling of pathogen inhibition.

Toxicity Studies:

Microorganism & Growth Media: In the present study, *E. coli* culture was maintained in nutrient agar media and was stored at 4.±1°C. The culture was inoculated in nutrient broth with concentrations varying from 2g/L to 10 g/L and methanol root extracts with concentrations varying from 10 g/L to 30 g/L respectively. The cultures were kept under shaking conditions at 37 °C. The biomass (g/L) was harvested at different time intervals and was compared with respect to the control conditions (0 g/L extract).

Kinetic Approach: The relationship between the specific growth rate and substrate concentration in absence of inhibitory substance can be described by the Monod equation as given below.

$$\mu = \frac{\mu_m}{1+\frac{K_s}{S}} \quad \text{..... (1)}$$

Where S is the substrate concentration (g l^{-1}), μ and μ_m are the specific growth rate and the maximum specific growth rate of microorganism (h^{-1}), respectively and K_s is the saturation constant (gl^{-1}), Eq. (1) can be linearized in double-reciprocal form:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{Ks}{\mu_m}\frac{1}{S} \quad \text{..... (2)}$$

K_s and μ_m values can be determined from the plot of 1/ μ versus $1/S_o$ (assuming S= S_o at the beginning of exponential growth) yields a linear line with a slope of K_s/ μ_m and y-axis intercept of 1/ μ_m. In presence of inhibitory (toxic) substances in nutrient media, microbial growth becomes inhibited, and specific growth rate depends on inhibitor concentration[12]. Inhibitions models viz. Non competitive and Uncompetitive are classified according to the effects of toxic compounds on the specific growth rate and saturation constant K_s and the rate expressions of the models are expressed as follows:

Non-competitive inhibition:

$$\mu = \frac{\mu_m}{\left[1+\frac{K_s}{S}\right]\left[1+\frac{C_0}{K_I}\right]} \quad \text{..... (3)}$$

Or;

$$\mu = \frac{\mu_{m,app}}{1+\frac{K_s}{S}} \quad \text{..... (4)}$$

Where;

$$\mu_{m,app} = \frac{\mu_m}{1+\left[\frac{C_0}{K_I}\right]} \quad \text{..... (5)}$$

Where; C_o and K_I are the methanol root extract concentration (g/L) and inhibition constants of extract (g/L), respectively. Eq. (3) can be linearized as similar to Eq. (2):

$$\frac{1}{\mu} = \frac{1}{\mu_{m,app}} + \frac{K_s}{\mu_{m,app}}\frac{1}{S} \quad \text{..... (6)}$$

The $\mu_{m,app}$ values can be determined from the linear plot of 1/ μ versus 1/S at different initial extract concentrations and then the value K_I can be calculated from Eq. (5).

Non-competitive inhibition model describing the extract inhibition was selected for assessing the dynamic behaviour of *E.coli* cells [13, 14].

Uncompetitive inhibition:

$$\mu = \frac{\mu_m S}{\left[\frac{K_s}{\left[1+\frac{C_0}{K_I}\right]} + S\right]\left[1+\frac{C_0}{K_I}\right]} \quad \text{..... (7)}$$

This equation can be linearized as follows;

$$\frac{1}{\mu} = \frac{1}{\mu_{m,app}} + \frac{K_s}{\mu_m}\frac{1}{S} \quad \text{..... (8)}$$

Where;

$$\mu_{m,app} = \frac{\mu_m}{1+\left[\frac{C_0}{K_I}\right]} \quad \text{..... (9)}$$

RESULT AND DISCUSSION:

Toxicity Studies: Influence of concentration of methanol extracts of root on growth pattern of *Escherichia coli* was studied. The growth pattern of *Escherichia coli* as a function of initial methanol root extract concentration was carried out by varying the extract concentration (0, 10, 20, 30g/L) in 2, 4, 6, 8, 10g/L nutrient broth with optimized pH values. The growth curves are shown in **Fig. 1-5** respectively. A decrease in biomass with an increase in extract concentration was seen at all concentrations of nutrient broth.

Minimum growth was noted in case of 30 g/L extract concentration at all concentrations of nutrient broth as compared to that of 10 g/L and 20 g/L extract concentration. Longest lag phase of 8 hrs at 30 g/L extract concentration and 2 g/L nutrient broth concentration was noted. Shortest lag phase of 3 hrs at 6,8 and 10 g/L of nutrient broth concentrations and 0 g/L of extract concentration was noted. The decrease in biomass at increasing concentrations of extract was caused due to toxicity of extract on *Escherichia coli* [15].

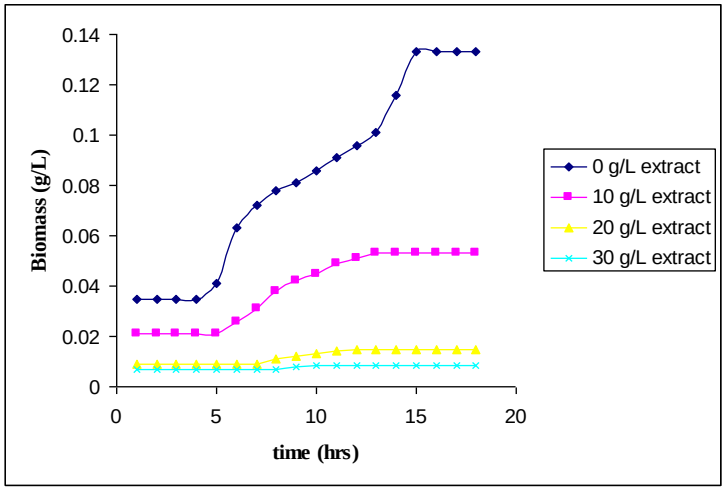

FIG 1: GROWTH PATTERN OF *ESCHERICHIA COLI* GROWN IN NUTRIENT BROTH (2g/L) AS A FUNCTION OF METHANOL ROOT EXTRACT CONCENTRATIONS RANGING FROM 10-30g/L

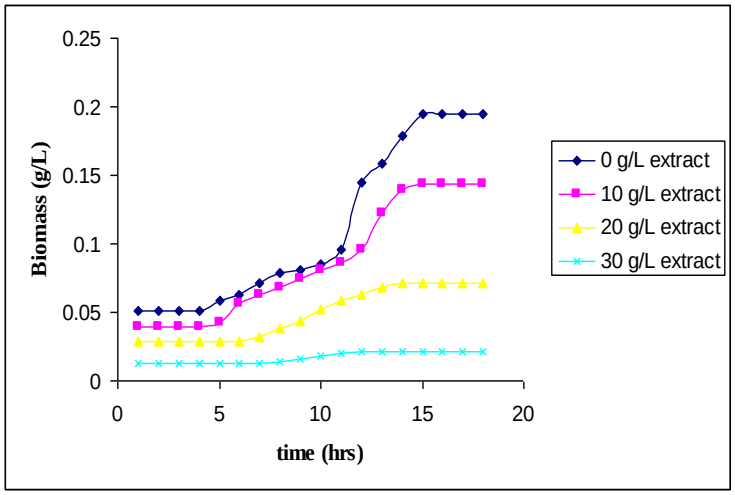

FIG 2: GROWTH PATTERN OF *ESCHERICHIA COLI* GROWN IN NUTRIENT BROTH (4g/L) AS A FUNCTION OF METHANOL ROOT EXTRACT CONCENTRATIONS RANGING FROM 10-30g/L

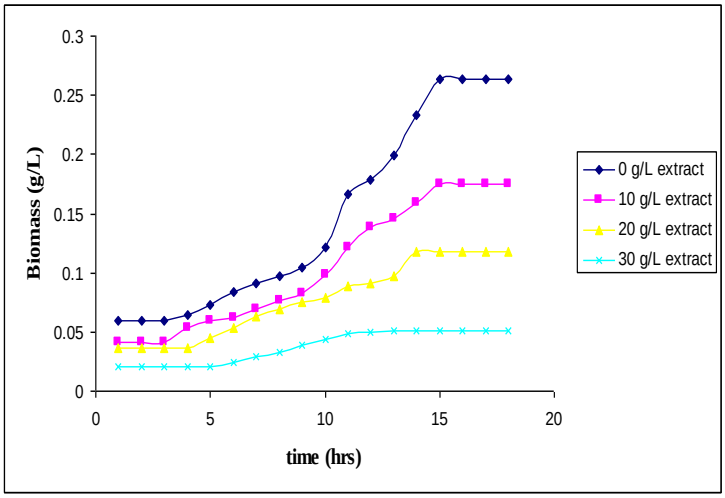

FIG. 3: GROWTH PATTERN OF *ESCHERICHIA COLI* GROWN IN NUTRIENT BROTH (6g/L) AS A FUNCTION OF METHANOL ROOT EXTRACT CONCENTRATIONS RANGING FROM 10-30g/L

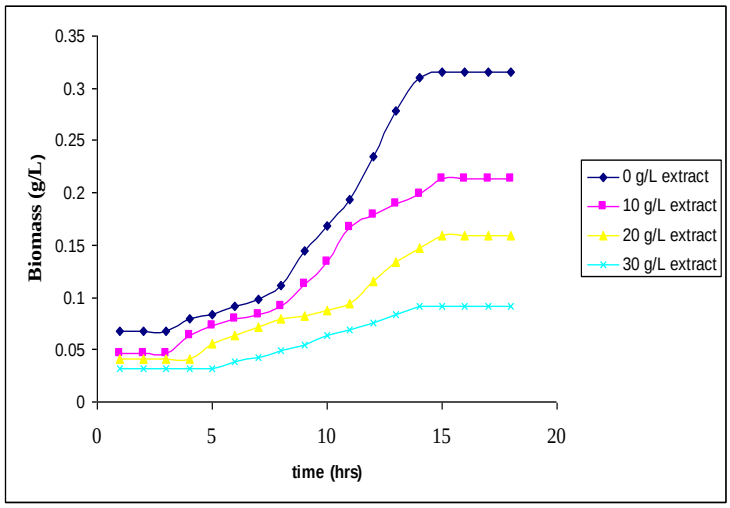

FIG. 4: GROWTH PATTERN OF *ESCHERICHIA COLI* GROWN IN NUTRIENT BROTH (8g/L) AS A FUNCTION OF METHANOL ROOT EXTRACT CONCENTRATIONS RANGING FROM 10-30g/L

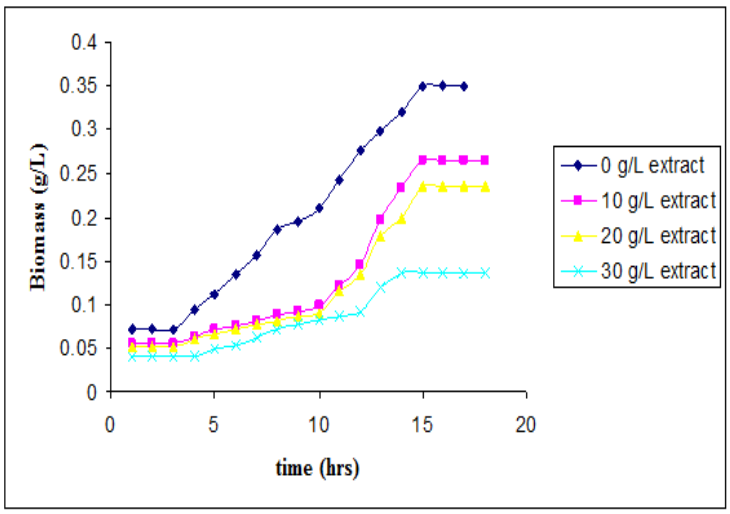

FIG. 5: GROWTH PATTERN OF *ESCHERICHIA COLI* GROWN IN NUTRIENT BROTH (10g/L) AS A FUNCTION OF METHANOL ROOT EXTRACT CONCENTRATIONS RANGING FROM 10-30g/L

Inhibition Models: In absence of Methanol root extract concentration, the values of μ_m and K_S for *Escherichia coli* was determined as 0.136 h^{-1} and 0.484 g/L. respectively from Monod equation by linear regression method. As the Monod expression for growth kinetics did not represent the inhibitory effects of the extract, Non competitive inhibition model and Uncompetitive model were tested to characterize the methanol root extract inhibition kinetics of *Escherichia coli*. The μ_{mapp} values could be determined from the intercept of linear plot of 1/ μ versus 1/S at different extract concentrations **(Fig. 6)**.The values of inhibition constant K_I was also estimated from found μ_{mapp} and known extract concentration values.

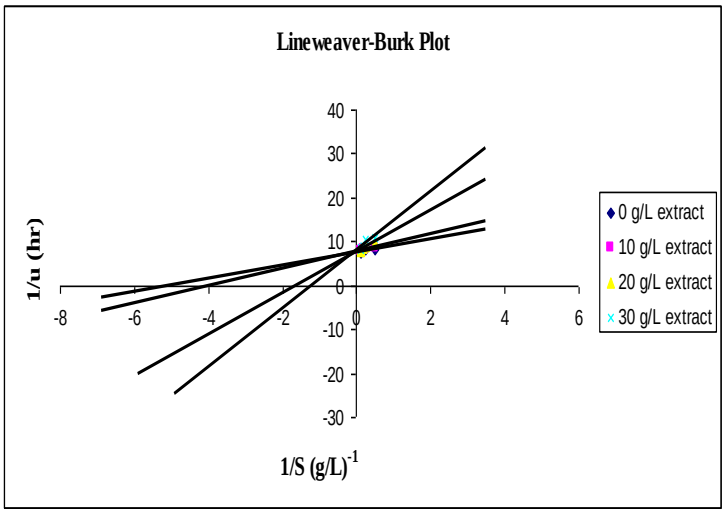

FIG. 6: LINEWEAVER-BURK PLOT FOR METHANOL EXTRACT OF ROOT OF *ALSTONIA SCHOLARIS*

Table 1 and 2 showed the values of Non competitive and Uncompetitive model parameters. The values of inhibition constant indicate the tolerance limit of *Escherichia coli* species. The inhibition constant values were obtained as 184.29, 180.35, 176.59g/L for 10, 20, 30g/L of extract concentration in case of both Non competitive and Uncompetitive inhibition models which confirmed the inhibitory effect of extract on growth of *Escherichia coli*. As parameters of both the models (Non competitive and Uncompetitive) showed similar response in terms of μ_{mapp} and K_I and almost similar response in case of K_S, Non competitive inhibition model was selected [12].

TABLE 1: NON COMPETITIVE INHIBITION MODEL PARAMETERS

Concentration (g/L)	μ_{mapp} $(hr)^{-1}$	K_S (g/L)	K_I (g/L)
10	0.129	0.539	184.29
20	0.122	0.634	180.35
30	0.116	0.729	176.69

TABLE 2: UNCOMPETITIVE INHIBITION MODEL PARAMETERS

Concentration (g/L)	μ_{mapp} $(hr)^{-1}$	K_S (g/L)	K_I (g/L)
10	0.129	0.562	184.29
20	0.122	0.674	180.35
30	0.116	0.745	176.69

CONCLUSION: Growth profile of *Escherichia coli* cells was obtained in presence of extract ranging from 10 to 30 g/L concentration. The cultures were prepared in nutrient broth ranging from 2 g/L to 10 g/L. The growth was found to decrease with an increase in extract concentration. Longest lag phase of 8 hrs was obtained

at 2 g/L nutrient broth concentration and 30 g/L Methanol extract concentration.

Monod Kinetics was used to calculate maximum specific growth and saturation constant values of 0.136 h^{-1} and 0.484 g/L. Two inhibition models namely Non competitive and Uncompetitive models were used to confirm the toxic effects of Methanol root extracts on *Escherichia coli*. The toxicity parameters were calculated from Lineweaver Burk plot. A decrease in specific growth rate and inhibition constant values and an increase in saturation constant values confirmed the toxic effects on *E. coli* cells.

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