3.3 RESULTS

3.3.1 Growth curve analysis of *Lactococcus garvieae* (Table 1; Graph 1).

The growth curve was measured by taking the optical density of culture broth at every six hour interval of time and O.D was found to increase as the time of incubation increase, it shown that there is steady growth of *L. garvieae* in the media.

3.3.2 Production of bacteriocin with different media

3.3.2.1 Man, Rogosa and Sharpe (MRS) medium (Table 2; Graph 2).

The *L. garvieae* was inoculated in MRS broth, consequently released bacteriocin level was evaluated which showed that at 48, 52, 56 and 60 hrs. of incubation *L. garvieae* yielded 12064.5, 3219.0, 2119.3 and 6454.4 AU/ml of bacteriocin level in MRS medium.

3.3.2.2 M17 medium (Table 3; Graph 3).

The *L. garvieae* was inoculated in M17, thus released bacteriocins level was evaluated which shows that at 48 h of incubation *L. garvieae* yielded 16454.4, 17449.5, 13219.0 and 17449.5 AU/ml of bacteriocin level in respectively.

3.3.2.3 Soybean casein digests broth (Table 4; Graph 4).

The *L. garvieae* was inoculated in Soybean casein digests broth, thus released bacteriocins level was evaluated which shows that at 48 h of incubation *L. garvieae* yielded 11464.5, 12119.3, 16363.5 and 17449.5 of bacteriocin level in Soybean casein digests broth medium.

3.3.2.4 Brain heart infusion broth (Table 5; Graph 5).

The *L. garvieae* was inoculated in M17, thus released bacteriocins level was evaluated which shows that at 48 h of incubation *L. garvieae* yielded 14363.6 AU/ml 12119.3, 13064.5, 15363.4 and 16363.5 of bacteriocin level in M17 medium.
3.3.3 Production of bacteriocin by *L. garvieae* with supplementation of various media sources

3.3.3.1 Carbon sources (Table 6; Graph 6).

The effect of the carbon source on cell growth and bacteriocin production was determined using M17 medium supplemented with 1% of different carbon sources. There was significantly higher bacteriocin production was observed in media containing lactose when compared to control and moderate production was observed in sucrose, fructose and raffinose viz; 8166.23, 6430.2, 7187.2 and 7191.47, pH of 5.63, 5.56, 6.23 and 5.60 and cell dry weight (CDW) of 1.52, 1.546, 0.640, 1.42 g l\(^{-1}\). Whereas, in glucose, xylose, galactose and arabinose there is moderate bacteriocin production was noticed that is 4239.8, 4170.1, 4855.8 and 3855.5 AU/ml and the pH was found to be 5.63, 6.06, 6.10, 6.40 and cell dry weight was found to be 1.413, 0.6600, 0.8732, 0.8633 respectively, and in maltose there was significantly lower bacteriocin production was observed that was found to be 3478.3 AU/ml and pH of 6.13 and the cell dry weight was found to be 0.76 g l\(^{-1}\) respectively.

3.3.3.2 Nitrogen sources (Table 7; Graph 7).

The effect of the Nitrogen source on cell growth and bacteriocin production was determined using M17 medium supplemented with 1% of different Nitrogen sources. The bacteriocin activity in control was found to be 14224.0 AU/ml, pH 6.4 and cell dry weight of 0.31 g l\(^{-1}\). There was significantly higher bacteriocin production was observed in media containing tryptone when compared with control and moderate production in yeast extract, soytone, casein was found to be 8188.0, 7318.2, 13390.0 and 7196.2 AU/ml, pH of 5.5, 6.2, 5.5 and 6.03 and cell dry weight (CDW) of 1.51, 1.57, 0.61 and 0.726 respectively. Whereas, in peptone there is significantly lower bacteriocin production was observed that was found to be 5649.5 AU/ml and
moderate bacteriocin production was observed in beef extract and casitone that was found to be 6230.6 and 6103.2 AU/ml the pH was found to be 5.6 and 6.1. Whereas, cell dry weight was found to be 1.39 and 0.79 g l\(^{-1}\) respectively.

### 3.3.3.3 Lactose (Table 8; Graph 8)

The effect of lactose concentration on the activity of bacteriocin was evaluated by adding 1 gm to 5 gm lactose to the media. The bacteriocin activity in control was found to be 13999.0 AU/ml, pH of 6.5 and the cell dry weight 0.31 g l\(^{-1}\).

The bacteriocin activity with supplementation of 1 gm of lactose was found to be significantly lowest that is 4136.8 AU/ml, pH of 5.7 and the cell dry weight 1.41 g l\(^{-1}\). Further, in 1.5 gm lactose supplementation the bacteriocin activity was found to be 4690.7 AU/ml, pH of 5.7 and the cell dry weight 1.50 g l\(^{-1}\). Additional, in 2 gm lactose supplementation the bacteriocin activity was found to be 4172.5 AU/ml, pH of 5.7 and the cell dry weight 1.54 g l\(^{-1}\).

When supplemented with 2.5 gm lactose the bacteriocin activity was found to be 5641.2 AU/ml, pH of 6.167 and the cell dry weight 0.653 g l\(^{-1}\) and in 3.0 gm lactose supplementation the bacteriocin activity was found to be 7181.2 AU/ml, pH of 6.2 and the cell dry weight 0.63 g l\(^{-1}\) and in 3.5 gm lactose supplementation the bacteriocin activity was found to be 8067.7 AU/ml, pH of 6.2 and the cell dry weight 0.79 g l\(^{-1}\) then in 4.0 gm lactose supplementation the bacteriocin activity was found to be 9990.0 AU/ml, pH of 5.7 and the cell dry weight 0.7667 g l\(^{-1}\) and in 4.0 gm lactose supplementation the bacteriocin activity was found to be 12971.0 AU/ml, pH of 5.6 and the cell dry weight 0.887 g l\(^{-1}\). Finally, in 4.5 gm lactose supplementation the bacteriocin activity was found to be 12971.0 AU/ml, pH of 5.6 and the cell dry weight 0.887 g l\(^{-1}\). There was significantly higher bacteriocin production was noticed in 5 gm lactose.
lactose fed medium as compared with control, the values were found to be 13979.0 AU/ml, pH of 5.2 and cell dry weight of 1.37 g⁻¹.

The lactose was an important carbon source that was needed all fundamental physiological reactions. The addition of lactose at an optimum level yielded good ample amount of bacteriocin from L. garvieae.

3.3.9 Yeast extract (Table 9; Graph 9).

The effect of Yeast extract on the activity of bacteriocin was evaluated by adding 1 gm to 4 gm Yeast extract to the media. The bacteriocin activity in control was found to be 1269.0 AU/ml, pH 6.49 and the cell dry weight 1.18 g⁻¹.

The bacteriocin activity with supplementation of 0.5 gm of Yeast extract was found to be 7699.4 AU/ml, pH of 6.1 and the cell dry weight 1.1 g⁻¹. The bacteriocin activity was found to be significantly lower at supplementation of 1 gm of tryptone added medium when compared with control and found to be 4063.2 AU/ml, pH of 5.27 and the cell dry weight 1.1 g⁻¹. The bacteriocin activity at supplementation of 1.5 gm of Yeast extract was found to be 4282.1 AU/ml, pH of 6.04 and the cell dry weight 1.88 g⁻¹ and at 2 gm of Yeast extract the activity was found to be 5992.2 AU/ml, pH of 6.21 and the cell dry weight 1.70 g⁻¹. The bacteriocin activity at supplementation of 2.5 gm of yeast extract was found to be 7939.0 AU/ml, pH of 5.17 and the cell dry weight 1.68 g⁻¹ and at 3 gm of yeast extract there was significant higher activity was noticed and found to be 12220.0 AU/ml as compared with control, pH of 5.62 and the cell dry weight 1.80 g⁻¹. The bacteriocin activity at supplementation of 3.5 gm of Yeast extract was found to be 9634.3 AU/ml, pH of 5.33 and the cell dry weight 1.80 g⁻¹ and at 4 gm of tryptone was found to be 12048.0 AU/ml, pH of 4.39 and the cell dry weight 1.90 g⁻¹.
Therefore, the addition of Yeast extract into the M 17 media enhanced the production of bacteriocin from *L. garvieae*.

### 3.2.9 Tryptone (Table 10; Graph 10).

The effect of tryptone concentration on the activity of bacteriocin was evaluated by adding 1 gm to 5 gm tryptone to the media. The bacteriocin activity in control was found to be 11994.0 AU/ml, pH of 6.43 and the cell dry weight 0.26 g l\(^{-1}\).

The bacteriocin activity with supplementation of 1 gm of tryptone the bacteriocin production was found to be significantly lower that is 5193.3 AU/ml, pH of 5.67 and the cell dry weight 1.40 g l\(^{-1}\). The bacteriocin activity at supplementation of 1.5 gm of tryptone was found to be 5973.1 AU/ml, pH of 5.59 and the cell dry weight 1.39 g l\(^{-1}\). The bacteriocin activity at supplementation of 2.0 gm of tryptone was found to be 7663.2 AU/ml, pH of 5.43 and the cell dry weight 1.43 g l\(^{-1}\) and at 2.5 gm of tryptone the activity was found to be 6680.8 AU/ml, pH of 5.8 and the cell dry weight 0.63 g l\(^{-1}\).

The bacteriocin activity with supplementation of 3.0 gm of tryptone was found to be 10016.0 AU/ml, pH of 6.14 and the cell dry weight 0.55 g l\(^{-1}\). The bacteriocin activity at supplementation of 3.5 gm of tryptone was found to be 8179.3 AU/ml, pH of 5.97 and the cell dry weight 0.76 g l\(^{-1}\). Further, supplementation of 4.0 gm of tryptone the bacteriocin production was found to be significantly higher that is found to be 11493.0 AU/ml, pH of 5.89 and the cell dry weight 0.87 g l\(^{-1}\).

The bacteriocin activity with supplementation of 5.0 gm of tryptone was found to be 7655.0 AU/ml, pH of 5.93 and the cell dry weight 0.79 g l\(^{-1}\). The bacteriocin activity in control was found to be 13999.0 AU/ml, pH of 6.54 and the cell dry weight
0.31 g\textsuperscript{-1}. The bacteriocin activity in control was found to be 13999.0 AU/ml, pH of 6.54 and the cell dry weight 0.31 g\textsuperscript{-1}.

Therefore, the addition of tryptone into the M 17 media enhanced the production of bacteriocin from \textit{L. garvieae}.

### 3.2.10 pH (Table 11; Graph 11)

The bacteriocin activity was evaluated at various pH from pH 2 to pH 10. The bacteriocin activity was significantly highest at pH 8 and pH 6, that is 8457.7 and 9992.0 AU/ml respectively and cell dry weight of 1.44 and 1.52 g\textsuperscript{-1} and optical density of 0.5667 and 0.5167 was noted at 600 nm. Whereas, there was lower activity at pH of 2, 4 and 10 i.e 4456.9, 5356.2, 652.37 AU/ml and cell dry weight was found to be less 0.3067, 1.35 and 0.56 g\textsuperscript{-1} and optical density was found to be 0.33, 0.3667 and 0.233 at 600 nm respectively.

The pH plays an important role in maintain the ionic balance of bacterial cell and physiological state of bacteria. Thus, maintenance of optimum pH was required for bacteriocin production.

### 3.2.11 Temperature (Table 12; Graph 12)

The bacteriocin activity was evaluated at various temperatures of 20, 25, 30, 35, 40 and 45°C. The bacteriocin activity was significantly highest at temperature of 40 and 45°C and activity of 11774.0 and 12629.0 AU/ml respectively and cell dry weight was found to be 0.61 0.56 g\textsuperscript{-1} and pH was found to be 5.62 and 6.28. The bacteriocin activity was found to be lowest at temperature of 20, 25, 30 and 35 °C such as 8623.6, 9988.3, 7305, and 9981.7 AU/ml and cell dry weight was seem to be
0.293, 1.35, 1.49, 1.42 and the pH was found to be 6.44, 5.69, 5.48 and 5.54 respectively.

Therefore, the present study revealed that the M 17 media was found to be the best media for the bacteriocin production and its production can be enhanced by adding various media constituents in requisite manner.

3.2.11 Comparison of components of M 17 medium and the optimized M 17 medium.

The optimization of medium for bacteriocin production was since been practiced for enhanced production. The components which govern the main role as substrates were added to the M 17 media and with changing concentrations of various components the desirable bacteriocin production was achieved. The glucose was omitted from optimized M 17 medium and sucrose, raffinose, casein and soytone was included which yielded better production of bacteriocin.

Therefore, the present results revealed that the M17 medium was found to be ideal medium for bacteriocin production.