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Curcuma Longa Extract Increases Growth of Salmonella Group of Species

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ABSTRACT

Curcuma longa is a widely used plant in folk medicine. In this study, we assessed the action of the ethanolic extract of *C. longa* on WHO strains of *Salmonella enterica* serotype Typhi and *Salmonella enterica* serotype Enteritidis. Curcumin is one of the most active ingredients of *C. longa*. In the presence of the ethanolic extract of *C. longa*, the bacterial count of both *Salmonella* was remarkably increased which was statistically highly significant. We also could not demonstrate MIC value even at 50 mg/ml concentration of the extract in *Salmonella enterica* serotype Typhi, while *Salmonella enterica* serotype Enteritidis only showed MIC value at a very high concentration of 6.25 mg/ml. However, two common bacteria *E. coli* and *Staphylococcus aureus* showed a MIC value of 0.39 and 3.125 mg/ml respectively. Thus, the use of *C. longa* in materials for public use should be done cautiously.

Keywords: *Curcuma longa*, Curcumin, antimicrobial action, minimum inhibitory concentration, *Salmonella enterica* serotype Typhi, *Salmonella enterica* serotype Enteritidis.

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INTRODUCTION

C. longa, commonly known as turmeric, is a perennial herbaceous plant belonging to the ginger family, Zingiberaceae. Native to South Asia, particularly India, it has been cultivated for thousands of years for its distinctive yellow-orange rhizomes, which are the primary source of the spice turmeric. Turmeric has been celebrated not only for its culinary applications but also for its extensive medicinal properties. The rhizomes of *C. longa* are rich in curcuminoids [1], the most notable being curcumin, which is renowned for its potent anti-inflammatory, antibacterial, antifungal, antioxidant, and antimicrobial activities. These properties have made turmeric a staple in traditional medicine systems such as Ayurveda and Traditional Chinese Medicine[2].

Studies have demonstrated curcumin's potential in managing inflammatory conditions, such as arthritis, and its possible protective effects against chronic diseases like cardiovascular disease, diabetes, and cancer. The carbon atoms of the carboxyl groups in curcumin are connected to the two ferulic acid molecules(Fig.1) by methylene bridge [3].

It has two aromatic ring systems containing o-methoxy phenolic groups. Curcumin is hydrophobic and poorly soluble in water but soluble in organic solvents like ethanol, dimethyl sulfoxide (DMSO), and acetone. Curcumin is relatively stable in acidic to neutral pH but degrades in alkaline conditions. Curcumin (Molecular formula: $C_{21}H_{20}O_6$) acts as a pro-oxidant in the presence of transition metal ions (Cu and Fe) and is a potent bio-protectant with a potentially wide range of therapeutic applications[4].

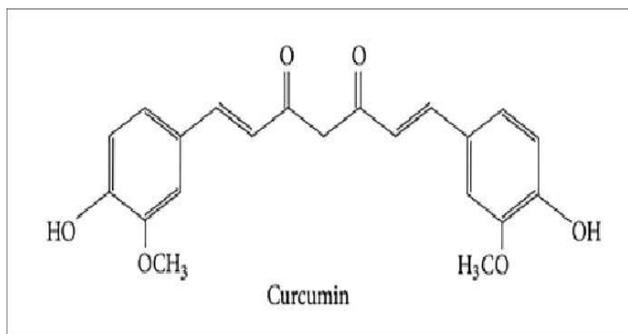


Figure 1: Molecular structure of curcumin.

***Salmonella enterica* serotype Typhi**

S. enterica serotype Typhi are bacteria that infect the intestinal tract and the blood. The disease is referred to as typhoid fever. Antibiotic treatment is required for *S. enterica* serotype Typhi infections. Without antibiotic treatment, the symptoms may last for months and the fatality rate can be as high as 20%. *S. enterica* serotype Typhi may cause initial constipation followed by pea soup offensive diarrhoea, continued high fever, headache, fatigue, loss of appetite, dizziness,

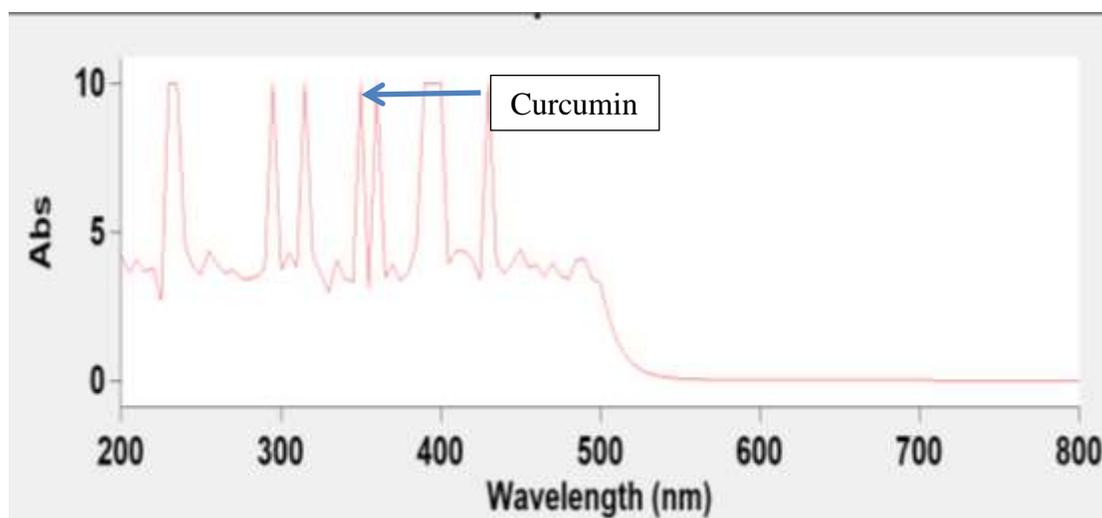
splenomegaly, leucopenia with relative lymphocytosis, relative bradycardia, and coated tongue[5].

Salmonella enterica serotype Enteritidis

S. enterica serotype Enteritidis, which is frequently associated with foodborne disease outbreaks, is one of the most common serotypes of *Salmonella* reported worldwide, and is a major source of intestinal infections in humans[6]. *S. enterica serotype Enteritidis* is the main sero type implemented in Salmonella food poisoning throughout the world, and is related to salmonellosis as they are transmissible[7].

Fluorescence activity

***C. longa*, specifically its active compound curcumin, exhibits notable fluorescence activity, which has been extensively studied and utilized in various scientific applications.** Curcumin exhibits strong fluorescence under ultraviolet (UV) light. The excitation maximum of curcumin is typically around 425 nm (Figure 2). The emission maximum occurs around 500-550 nm, resulting in a bright yellow-green fluorescence. The fluorescence intensity and emission spectra of curcumin can vary significantly depending on the solvent used. In polar solvents, curcumin tends to have higher fluorescence intensity. For instance, in ethanol, the fluorescence is more pronounced compared to non-polar solvents like hexane. The fluorescence of curcumin is attributed to its conjugated structure, which allows for efficient absorption and emission of light. The extended system of double bonds and aromatic rings in the curcumin molecule enables it to absorb UV light and re-emit it at a longer wavelength in the visible spectrum.



430.0	10.000
410.0	4.390
400.0	10.000
370.0	3.943
360.0	10.000
350.0	10.000
335.0	4.066
315.0	10.000
305.0	4.270
295.0	10.000
270.0	3.752
255.0	4.366
235.0	10.000
220.0	3.816
210.0	4.049

Figure 2: UV Spectra of Curcuma longa extract

MATERIALS AND METHOD

Raw turmeric was taken (Figure 3), washed thoroughly in sterile distilled water, chopped with a sterile knife and then 1 gm of it was weighed in a laboratory weighing balance. Then it was smashed to paste with the help of a pestle. To this 1 gm of raw curcumin extract 10 ml of ethanol was added and vortexed to mix them well. The solution was kept in incubation at room temperature for 48 hours (Figure 4). The solution was centrifuged and the supernatant was separated. In this study, the possible antibacterial action of ethanolic extract of *C. Longa* extract was tested against two WHO strains of Salmonella species (*S.enterica* serotype Typhi and *S.enterica* serotype Enteritidis)[8].



Figure 3: Raw Curcuma longa

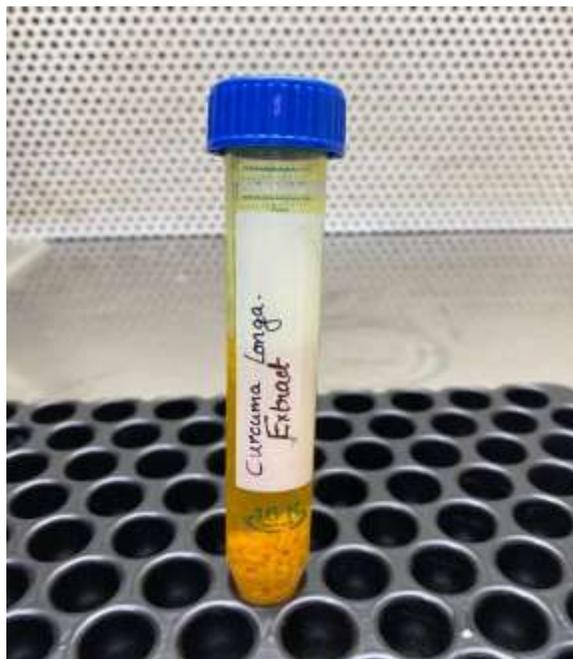


Figure 4: Ethanolic extract of *Curcuma longa*

Procedure of MIC(minimum inhibitory concentration) value determination

MIC is the lowest concentration of an antibacterial agent which, under strictly controlled in vitro conditions, completely prevents visible growth of the test strain of an organism[11]. A quantitative method of susceptibility testing, an MIC helps determine which class of antibiotic is most effective. This information can lead to an appropriate choice of an antibiotic that will increase chances of treatment success and help in the fight to slow antibiotic resistance[12].

A sterile microtitre plate was taken to which at first, the 100 μ L Mueller Hinton broth was added to each microwell. Then 100 μ L of the extract was added to the first well and then serially diluted along the horizontal row up to the eighth well, and then 100 μ L of the excess fluid was discarded from the eighth well. Thus in each step, there was a double dilution of the extract till the eighth well. After this 0.5 MacFarland[9] bacterial suspensions in a fixed quantity (10 μ L) were added to the wells in all eight wells and mixed. In another row, similar dilutions were made with alcohol as control and the bacterial suspensions were also similarly added. In different rows, different bacterial suspensions were added. The Optical Density of these wells was measured at 620 nm wavelength and subsequently, the plate was incubated overnight in a 37°C Incubator. The next day, the Optical Density was measured again, providing us 2 OD values, one at 0 hr and another at 24 hr[10]. The difference in the O.D. value (24hr-0hr) obtained was calculated and compared with the control for the determination of MIC values.

We also studied two common bacteria – *Escherichia coli* and *Staphylococcus aureus* to compare with *Salmonella* bacteria.

RESULTS AND DISCUSSION

C. Longa extract significantly promotes the growth of *Salmonella enteric* serotype Typhi without demonstrating MIC value even at 50 mg/ml concentration of the extract. *S.enterica* serotype Enteritidis only showed some antimicrobial activity in a high concentration of the extract (6.25 mg/ml), while inhibiting the growths of *E.coli* and *Staphylococcus aureus* with MIC Value of only 0.39 and 3.125 mg/ml respectively (Figure 5-8).

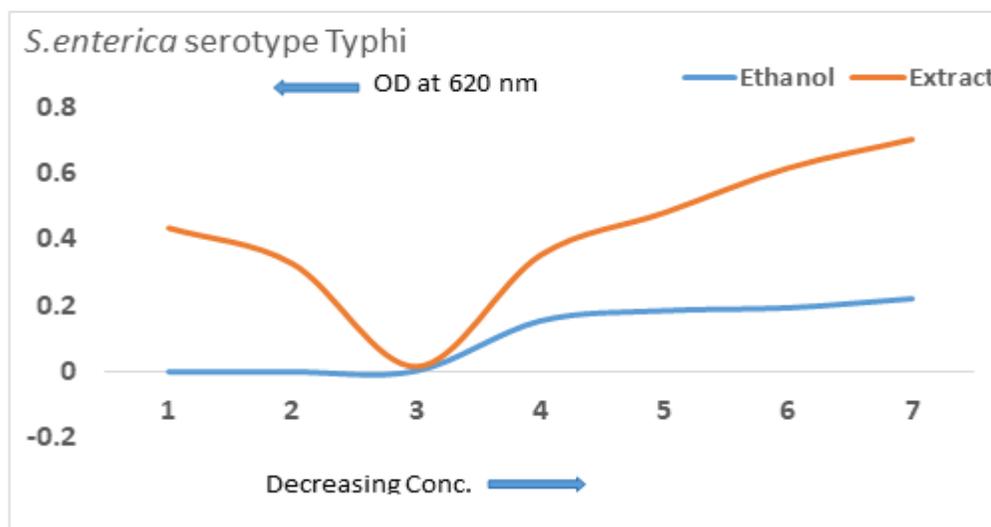


Figure 5: Showing effect of *C. longa* extract on the growth of *S. enterica* serotype Typhi. There is no inhibition of growth. 1: 50mg/ml, 2: 25 mg/ml, 3:12.5 mg/ml, 4: 6.25 mg/ml, 5:3.125 mg/ml. 6:1.56 mg/ml.7:0.78 mg/ml, 8:0.39 mg/ml.

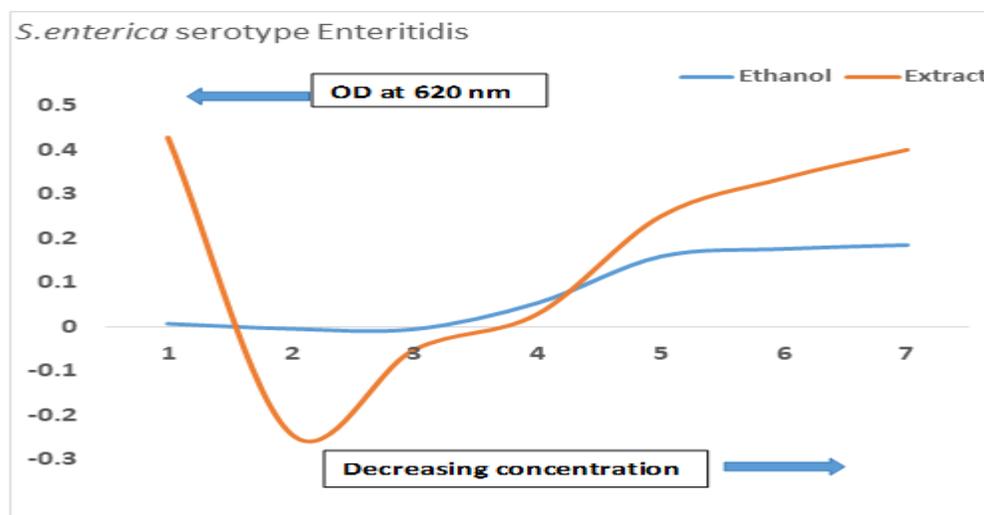


Figure 6: Showing effect of *C. longa* extract on the growth of *S. enterica* serotype Enteritidis. MIC value : 6.25 mg/ml.1: 50mg/ml, 2: 25 mg/ml, 3:12.5 mg/ml, 4: 6.25 mg/ml, 5:3.125 mg/ml. 6:1.56 mg/ml.7:0.78 mg/ml, 8:0.39 mg/ml..

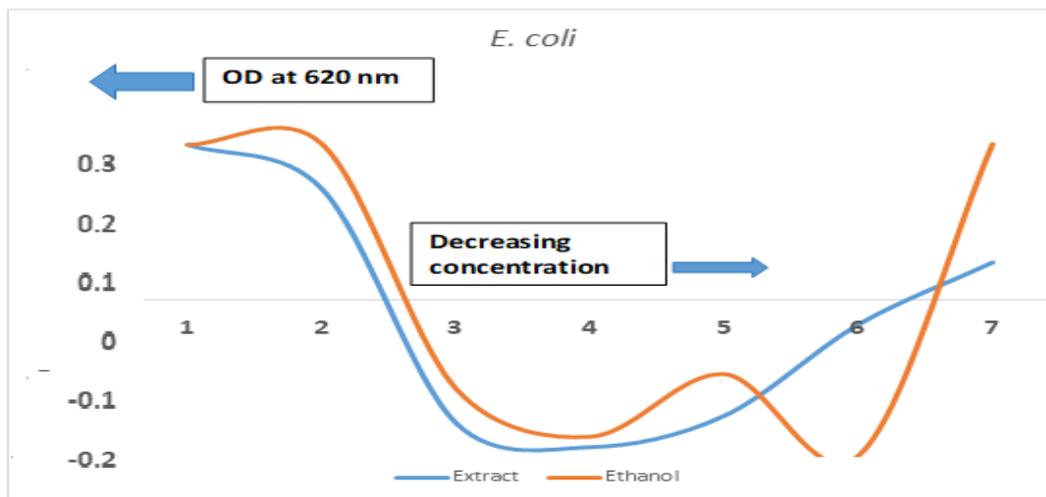


Figure 7: Showing effect of curcumin on the growth of *E.coli*. MIC value 0.39 mg/ml. 1: 50mg/ml, 2: 25 mg/ml, 3:12.5 mg/ml, 4: 6.25 mg/ml, 5:3.125 mg/ml. 6:1.56 mg/ml.7:0.78 mg/ml, 8:0.39 mg/ml..

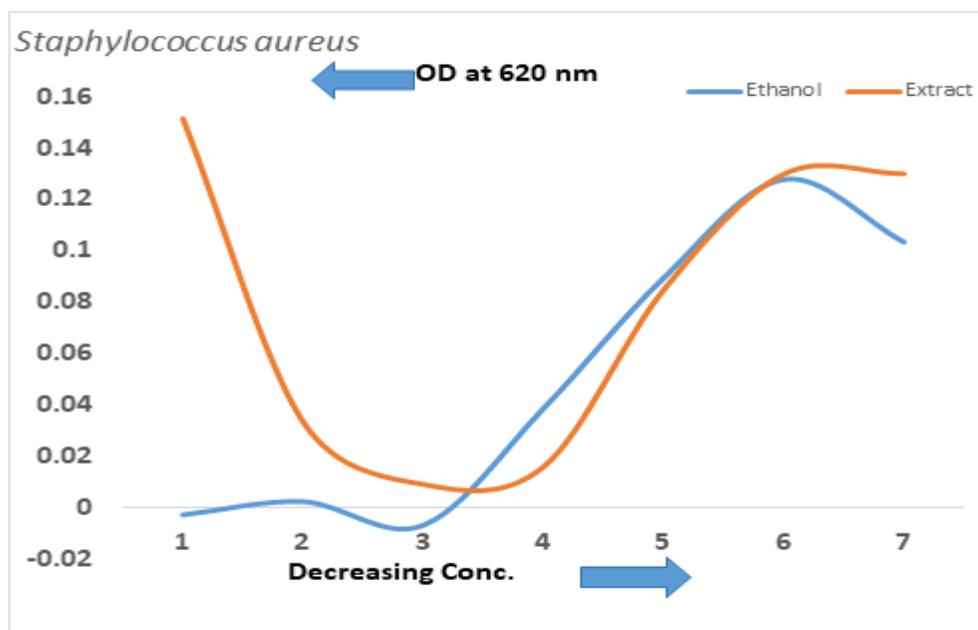


Figure 8: Showing effect of curcumin on the growth of *Staphylococcus aureus*. MIC value 3.125 mg/ml. 1: 50mg/ml, 2: 25 mg/ml, 3:12.5 mg/ml, 4: 6.25 mg/ml, 5:3.125 mg/ml. 6:1.56 mg/ml.7:0.78 mg/ml, 8:0.39 mg/ml..

For colony count (bacterial count) study (Figure 9-13) petri plates containing MH agar was streaked with *S.enterica* serotype Typhi and *S.enterica* serotype Enteritidis. There were plates of MH agar (20 ml) mixed with 200µL ethanolic *C. longa* extract before the plate was solidified and then streaked with *Salmonella* spp. to check whether the growth of *Salmonella* is inhibited or *Salmonella* is inhibited or promoted along with control plates without the extract. In both the plates

containing extract, the growth of *Salmonella* spp. was increased as compared to the plates having only MH agar.

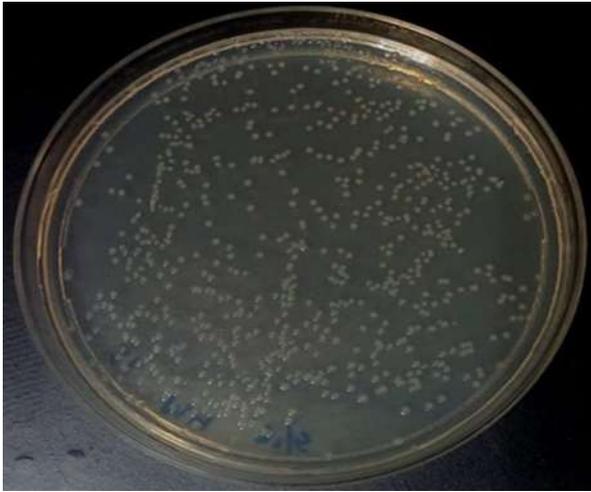


Figure 9: MH agar plate streaked with *S. enterica* serotype Typhi



Figure 10: MH agar+C. longa extract streaked with *S. enterica* serotype Typhi

Table 1: Colony count of *S. Enterica* serotype Typhi

<i>S. enterica</i> serotype Typhi	Control	With extract
Colony Count 1	712	1070
Colony Count 2	730	1150
Colony Count 3	760	1195
MEAN+SD+SEM	734±19.80±11.43	1138.33±51.70±29.84

Table 2: P value matrix (*S. enterica* serotype Typhi) using MedCalc statistical software

<i>S. enterica</i> serotype Typhi	
t value	12.650
P value	0.0002

The p value is 0.0002, which means the difference is highly significant at 0.0002 level.

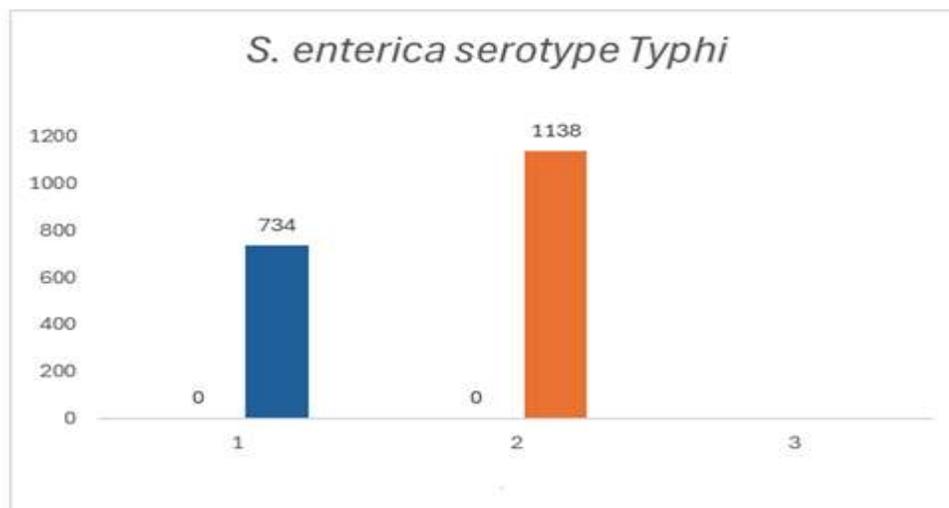


Figure 11: Average colony count of *S. enterica* serotype Typhi (Blue bar control, orange bar with extract).



Figure 12: MH agar plate streaked with *S. enterica* serotype Enteritidis [Control]

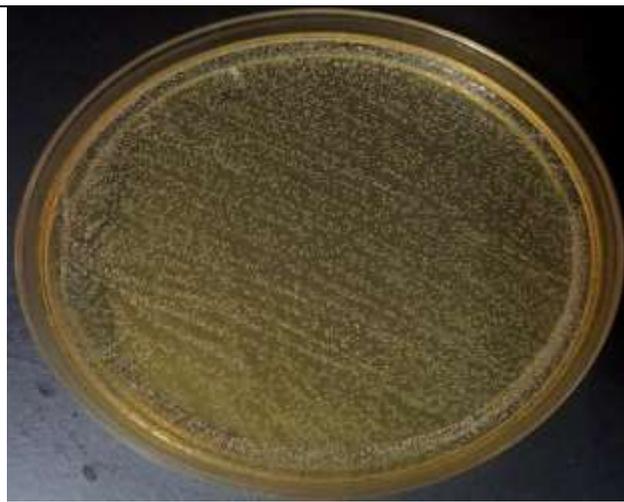


Figure 13: MH agar+C.longa extract plate streaked with *S. enterica* serotype Enteritidis

Table 3: Colony count of *S. enterica* serotype Enteritidis

<i>S. enterica</i> sero type Enteritidis	Control	With Extract
Colony Count 1	2154	3572
Colony Count 2	2172	3569
Colony Count 3	2187	3592
MEAN+SD+SEM	2171±13.50±7.79	3577.66±10.21±5.90

Table 4: P value matrix (*S. enterica* serotype Enteritidis) using Med Calc statistical software.

<i>S. enterica</i> serotype Enteritidis	
t value	143.94
P value	< 0.0001

P value less than 0.05 means significant. In this case, the p value is <0.0001, which means the difference is highly significant at < 0.0001 level.

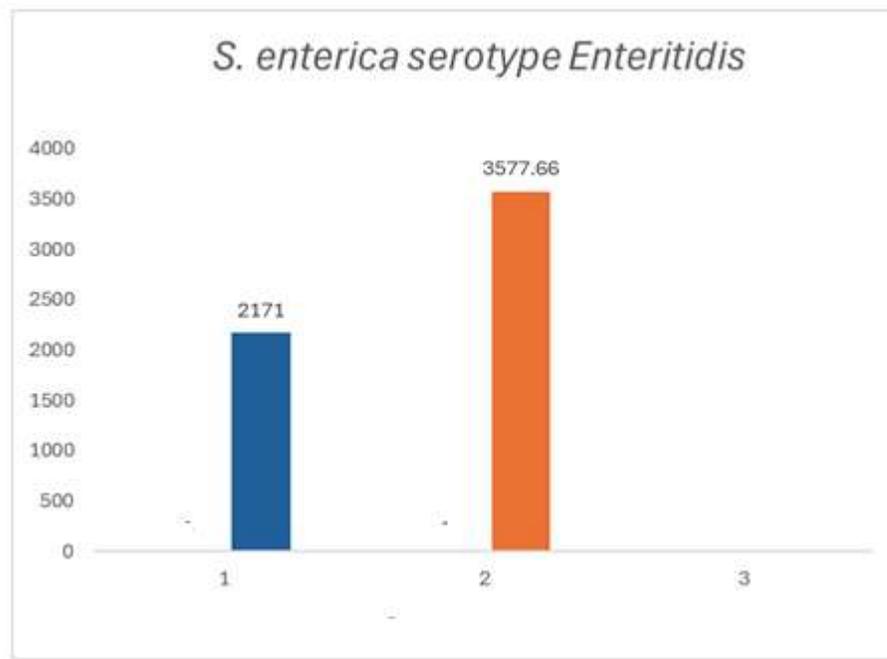


Figure 14: Average colony count of *S. enterica* serotype Enteritidis (Blue bar control, orange bar with extract).

DISCUSSION

Although curcumin is known to reduce the motility of *Salmonella enteric* serovar Typhimurium decreasing the number of flagella in them [13], in the murine model it increases pathogenicity [14]. It was found that curcumin increased the resistance of *Salmonella* against many antimicrobials, as well as reactive oxygen and nitrogen species. Upregulation of different genes *pmrD* and *pmrHFIJKLM*, *mntH*, *sodA* and *sitA* is responsible for this [14]. The up regulation of *sodA* and *sitA* genes is related to the iron chelation property of curcumin. Curcumin also down regulates SP 12 genes [14]. It also up regulates *Salmonella invA*, *fliC* and *siiE* genes which are known virulent genes [15]. Thus, increased growths in our study of *Salmonella* may be related to these factors.

CONCLUSION

As per the results observed, we can conclude that curcumin significantly promotes the growth of *Salmonella* bacteria. Thus for public use, curcumin should be judiciously used.

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AUTHOR'S CONTRIBUTION:

Dr.Satadal Das designed the study procedure, analysed the data and corrected the manuscript. Ms. Isha Majumder performed the experiment under the guidance of Mr.Arup Kumar Dawn. Dr. Bhaskar Narayan Chaudhari and Dr.Partha Guchait analysed the data and corrected the manuscript.

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