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Original Research Article

Direct and Sunlight-Induced Photosensitizing Antibacterial Activities of *Curcuma longa* Extract

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Abstract: *Curcuma longa* is a widely used plant in folk medicine. Here, we assessed the antimicrobial activities of the ethanolic extract of *Curcuma longa* with particular emphasis on its combined effect with sunlight, as the extract can produce fluorescence in the presence of UV rays. Curcumin is one of the most active ingredients of *Curcuma longa*. The ethanolic extract of *Curcuma longa* when added to lawn cultures of gram-positive and gram-negative bacteria and exposed to sunlight the growth of bacteria was remarkably reduced. Micro broth dilution assay also showed decreased MIC values after exposure to sunlight with MSSA. Our results encourage the potential use of *Curcuma longa* as an antibacterial product.

Keywords: *Curcuma longa*, Curcumin, antibacterial action, sunlight, UV ray.

INTRODUCTION

Curcuma longa (turmeric) is a flowering herb belonging to the Ginger family (Zingiberaceae). It is mostly of Indian origin and has been included in various traditional systems of medicine such as Ayurveda, also it has an extensive application in culinary as a spice or a food colouring agent. Curcumin is one of the most active ingredients of turmeric. Curcumin is a yellow-coloured low molecular weight, naturally polyphenolic compound found in the rhizome of *Curcuma longa* (turmeric). It has numerous benefits showing a wide range of pharmacological activities including antioxidant, anti-inflammatory, antiparasitic, antiviral, antimicrobial, anti-amyloid, and antitumor properties [1]. The carbon atoms of the carboxyl groups in curcumin are connected to the two ferulic acid molecules by methylene bridge [2]. Phenolic groups and conjugated double bonds (Fig 1) are also present in this lipophilic molecule. Chemically, curcumin can exist in tautomeric diketo and keto-enol forms. Curcumin incorporates a seven-carbon linker and three major functional groups.

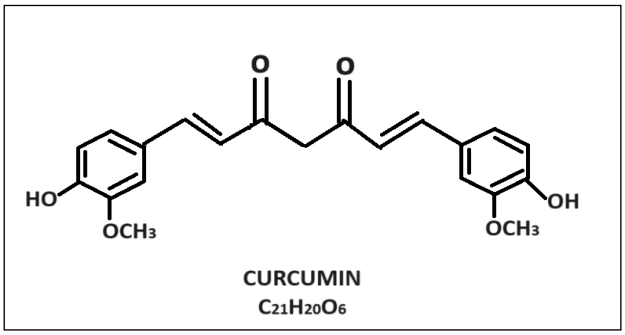

Fig 1: Molecular structure of curcumin.

Fluorescence activity

Curcumin can also produce fluorescence in the right conditions [3]. If curcumin is mixed into alcohol whilst illuminated by UV light, a bright green-yellow fluorescence can be seen (Fig 2). As curcumin is soluble in alcohol but not in water hence alcohol is used, and this

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enhances the property of fluorescence. The electrons in the curcumin molecules absorb the ultraviolet light, causing them to gain energy and move to an excited state due to which fluorescence occurs. Some of the extra energy is lost as vibrational energy, and then the electrons fall back to the ground state, emitting visible light as they do so. This gives the green-yellow glow, basically showing fluorescence. This polyphenolic molecule possesses a strong absorption band around 410 nm, and fluorescence emission between 460 and 550 nm. Curcumin also shows some phosphorescence, which demonstrates some ability to generate singlet oxygen and oxidize various cell structures, making it a potential photosensitizer [4]. We compared the antimicrobial activities of the extract in normal conditions and during its fluorescence under sunlight along with a control of exposed culture plate in ultraviolet rays.

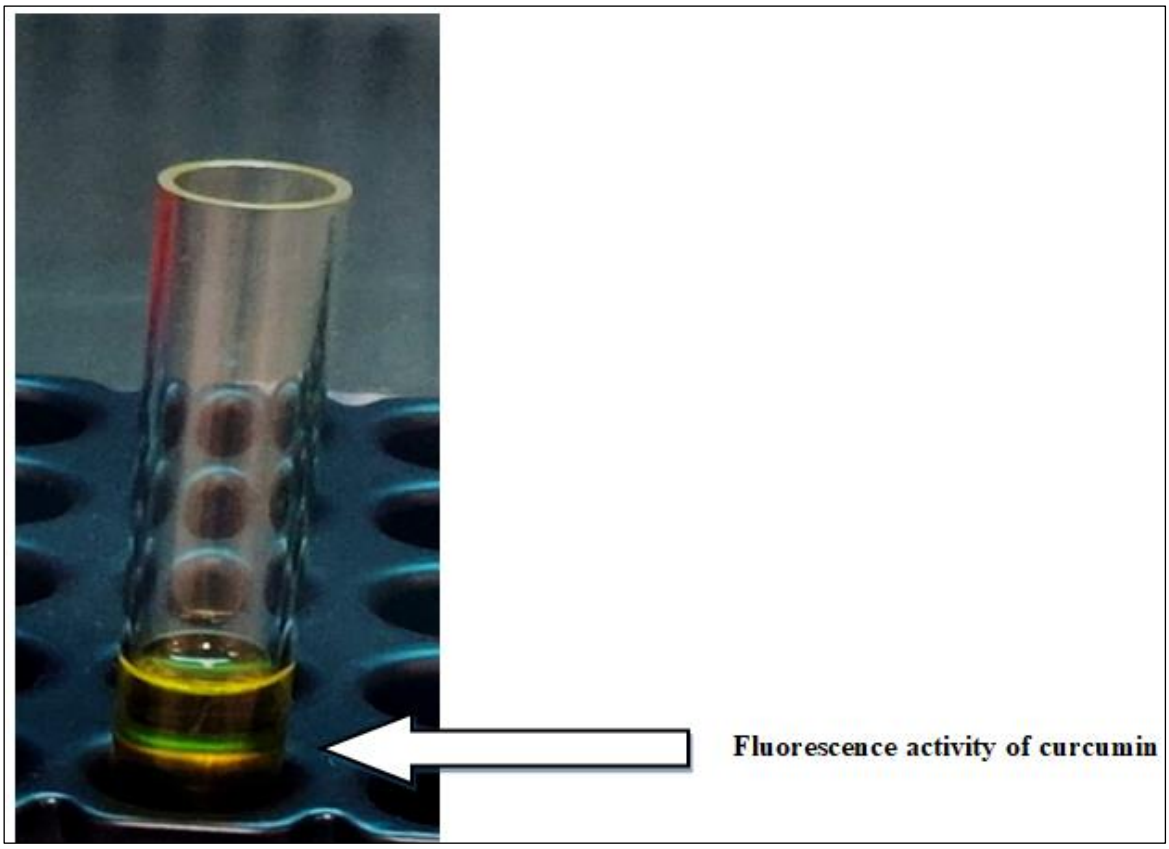

Fig 2: Fluorescence activity of *Curcuma longa*

MATERIAL AND METHODS

Raw turmeric was procured (Fig 3), washed thoroughly in tap water and then in sterile distilled water, chopped with a sterile knife and then 1 gm of it was weighed by a digital chemical balance. Then it was smashed to paste with the help of a pestle.10 ml of ethanol was measured accurately and previously weighed 1 gm of raw turmeric was mixed into the ethanol and mildly vortexed. The solution was kept in incubation at room temperature for 48 hours (Fig.4). Four petri plates were taken in each set of experiments and each plate was subjected to different experimental situations. In the first plate MH agar (Mueller Hinton agar) / Blood agar medium was placed in aseptic conditions in a biosafety cabin. Inoculums (0.5 MacFarland standard) of Methicillin-susceptible *Staphylococcus aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli*, and *Pseudomonas aeruginosa* were streaked over the medium in different plates and the bacteria were allowed to grow as control experimental sets for overnight. In another experimental set petri plates with MH agar were placed in sterile conditions and along with inoculums of MSSA, MRSA, *E. coli* and *Pseudomonas* spp. separately, the extract of *Curcuma longa* was added. The plates were then incubated at 37^{O}C overnight. Another experimental set was prepared as before and exposed under sunlight for 10 minutes and similarly incubated. Another control experimental set was prepared like the first control set which was exposed to UV rays for 10 minutes and similarly incubated. The plates were observed the next day and colony counts were performed. All the sets of experiments were repeated thrice and average results were calculated and statistically evaluated.

Determination of MIC values against the MSSA, MRSA, *E. coli* and *Pseudomonas* spp.

The bacterial isolates were made into 0.5 McFarland opacity bacterial suspensions using Normal Saline (NS) with DensiCHEK®. Normal Saline (NS)

was used to maintain the tonicity of the medium and to prevent the lysis of these bacterial cells. At first, the 100 µL Mueller Hinton broth was added to each microwell of a sterile microtitre plate. Then 100 µL 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 µL 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 bacterial suspensions in a fixed quantity (10 µL) were added to the wells in all eight wells. In another row, similar dilutions were made with vehicle alcohol as control and the bacterial suspensions were also similarly added. In this way, 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. The change in the O.D. value obtained was calculated and compared with the control for the determination of MIC values. Repeat experiments were also done after exposure to sunlight for 10 minutes.

Fig 3: Raw *Curcuma longa*

Fig 4: Ethanolic extract of *Curcuma longa*

RESULTS

The results of this study are given in Fig 5 to Fig 20 and in Table 1 to Table 8. The plate study showed a significant decrease in bacterial colony counts with all bacterial species after exposure to sunlight. In the study of MIC values, however, although direct curcumin without exposure to sunlight showed MIC values between 6.125 mg/ml to 12.5 mg/ml, after exposure to sunlight remarkable decrease in MIC value was only found with MSSA. This may be due to factors like poor penetration of UV-A rays in sunlight, interference of other fluorescent molecules as in *Pseudomonas*,

protection from UV-ray by the carotenoid pigment of MRSA etc.

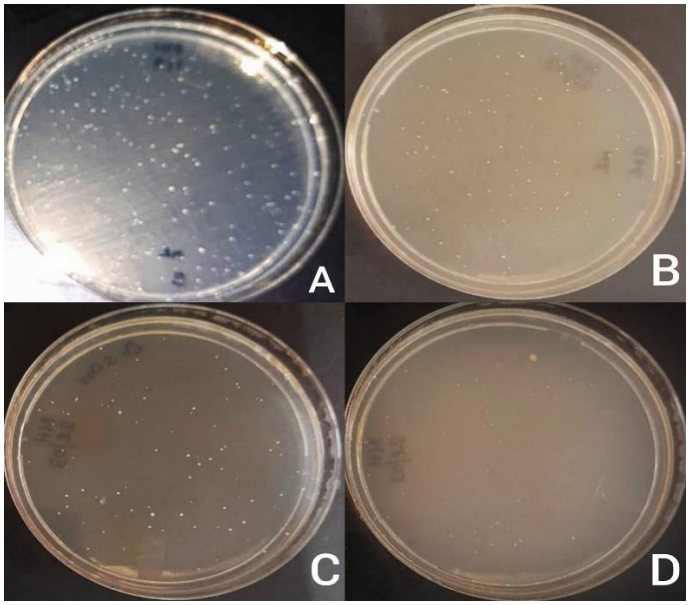

Fig 5: Effect of the extract, UV ray,sunlight on the growth of MRSA on MH plate. A: Control, B: Only extract, C: Extract and UV ray, D: Extract and sunlight

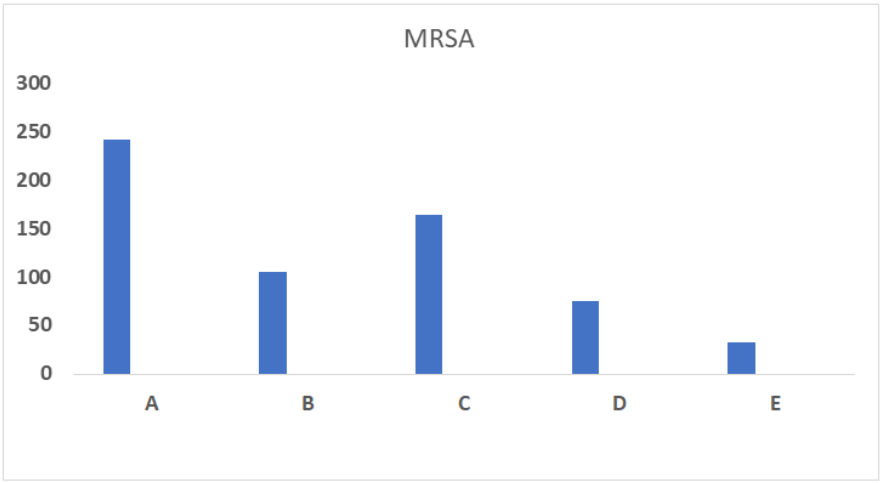

Fig 6: Average colony count of MRSA in different experimental sets. A: Control, B: only extract, C: Only sunlight, D: Extract and UV ray, E: Extract and sunlight

Table 1: Colony count of MRSA in different experimental sets

MRSA	Control	Only extract	Only sunlight	Extract+ UV ray	Extract+ sunlight
Colony count 1	247	92	165	74	30
Colony count 2	238	117	140	80	33
Colony count 3	242	105	188	70	35
MEAN+SD+SEM	242±4.51±2.60	105±12.50±7.22	164±24.05±13.86	74.66±5.03±2.91	32.66±2.51±1.45

Table 2: P value matrix (MRSA) between different experimental sets (MedCalc statistical software)

	Control	Only extract	Only sunlight	Extract +UV ray	Extract + Sunlight
Control	-	-	-	-	-
Only extract	0.0001	-	-	-	-
Only sunlight	0.0053	0.0196	-	-	-
Extract +UV ray	<0.0001	0.0176	0.0032	-	-
Extract + Sunlight	<0.0001	0.0006	0.0007	0.0002	-

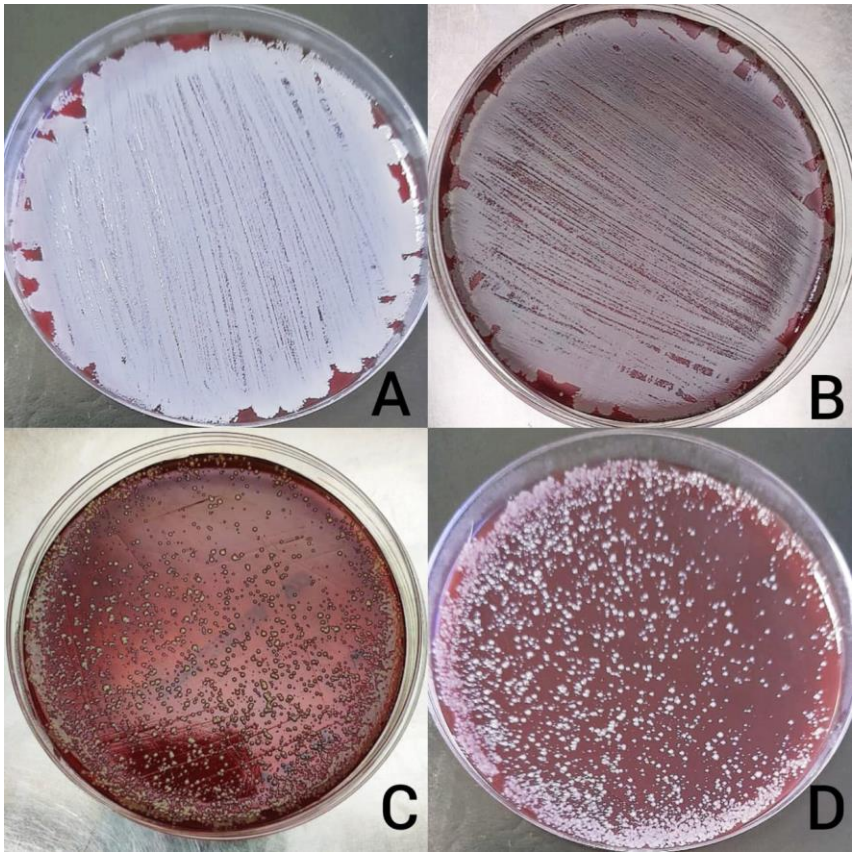

Fig 7: Effect of the extract, UV ray, sunlight on the growth of MSSA on blood agar plate. A: Control, B: Only extract, C: Extract and UV ray, D: Extract and sunlight

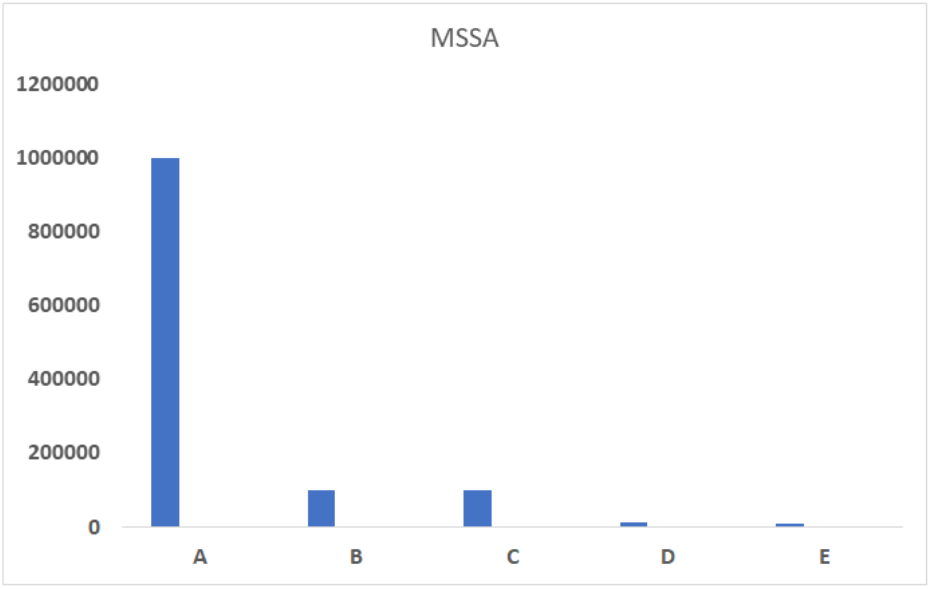

Fig 8: Average colony count of MSSA in different experimental sets. A: Control, B: only extract, C: only sunlight, D: extract and UV ray, E: Extract and sunlight

Table 3: Colony count of MSSA in different experimental sets

MSSA	Control	Only extract	Only sunlight	Extract+ UV ray	Extract+ sunlight
Colony count 1	~10^6	~105	≥10^5	≥10^4	7854
Colony count 2	~10^6	~10^4	≥10^5	≥10^4	7768
Colony count 3	~10^6	~10^4	≥10^5	≥10^4	7842
Mean±SD± SEM	1000000±13.75 ±7.94	100001.3±15.82 ±9.13	100001±15.10±8.72	9999±6.9±12.01	7821±46.58±26.89

Table 4: P value matrix (MSSA) between different experimental sets (MedCalc statistical software)

	Control	Only extract	Only sunlight	Extract +UV ray	Extract + Sunlight
Control	-				
Only extract	<0.0001	-			
Only sunlight	<0.0001	=1.000	-		
Extract +UV ray	<0.0001	<0.0001	<0.0001	-	
Extract + Sunlight	<0.0001	<0.0001	<0.0001	<0.0001	-

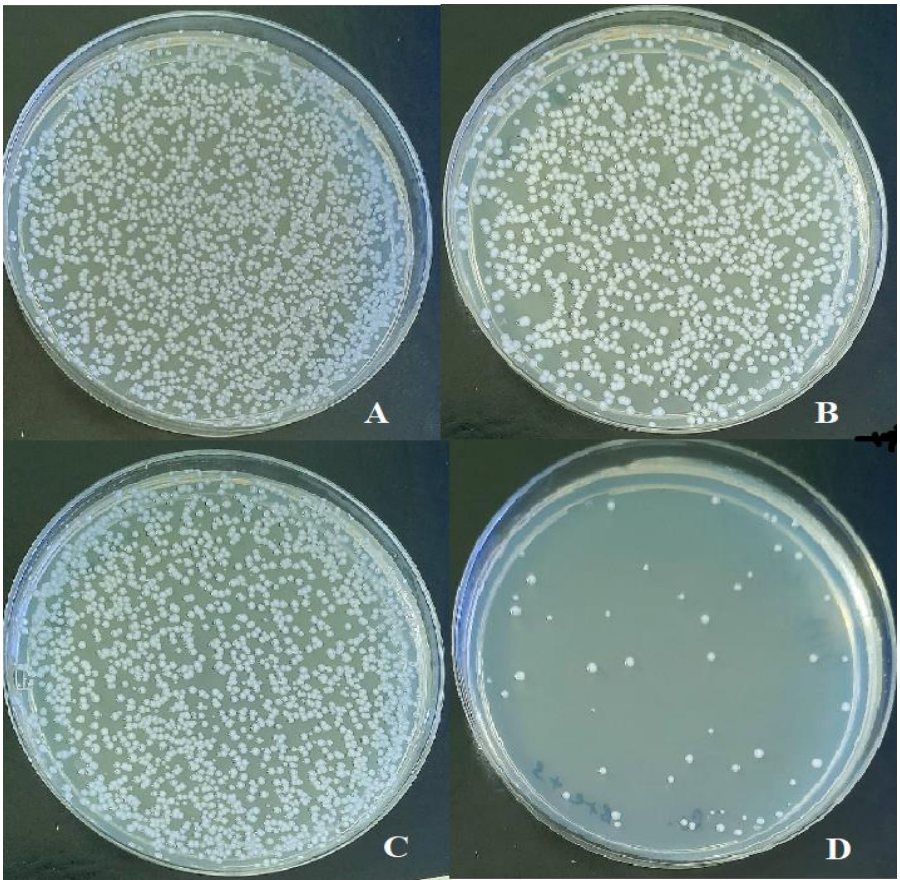

Fig 9: Effect of the extract, UV ray, sunlight on the growth of *E. coli* on M H plate. A: Control, B: Only extract, C: Extract and UV ray, D: Extract and sunlight

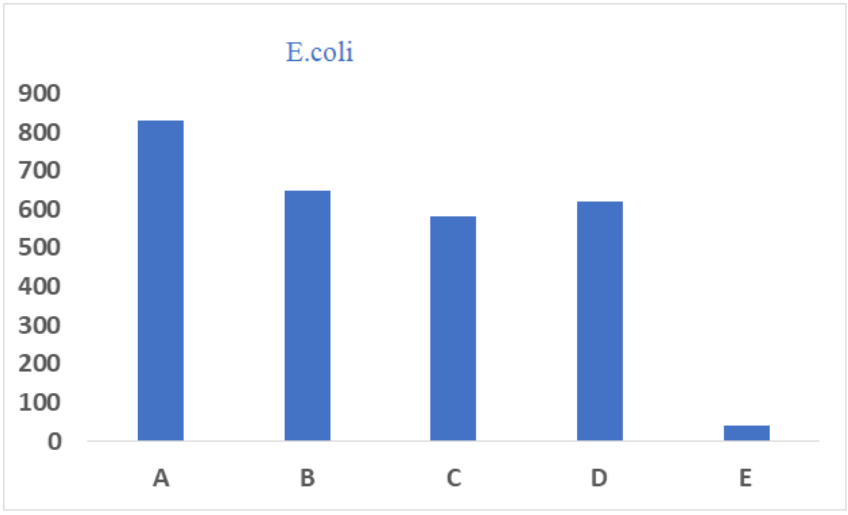

Fig 10: Average colony count of *E. coli* in different experimental sets. A: Control, B: only extract, C: Only sunlight, D: Extract and UV ray, E: Extract and sunlight

Table 5: Colony count of *E. coli* in different experimental sets

	Control	Only extract	Only sunlight	Extract +UV ray	Extract + Sunlight
Colony count 1	**832**	**728**	**568**	**560**	**40**
Colony count 2	**842**	**623**	**554**	**668**	**45**
Colony count 3	**810**	**596**	**628**	**635**	**34**
Mean±SD±SEM	**828±16.37±9.45**	**649±69.73 ±40.26**	**583.33±39.31 ±22.69**	**621±55.34±31.95**	**39.67±5.51±3.18**

Table 6: P value matrix (*E. coli*) between different experimental sets (MedCalc statistical software)

	Control	Only extract	Only sunlight	Extract +UV ray	Extract + Sunlight
Control	**-**				
Only extract	**0.0124**	**-**			
Only sunlight	**0.0006**	**0.2284**	**-**		
Extract +UV ray	**0.0034**	**0.6149**	**0.3909**	**-**	
Extract + Sunlight	**<0.0001**	**0.0001**	**<0.0001**	**0.0001**	**-**

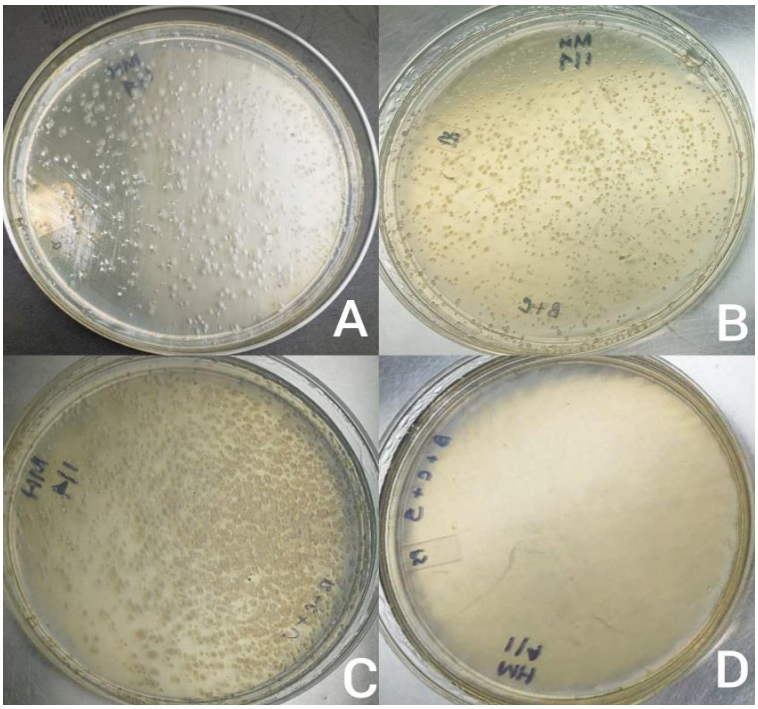

Fig 11: Effect of the extract, UV ray, sunlight on the growth of *Pseudomonas* spp. on MH agar plate. A: Control, B: Only extract, C: Extract and UV ray, D: Extract and sunlight

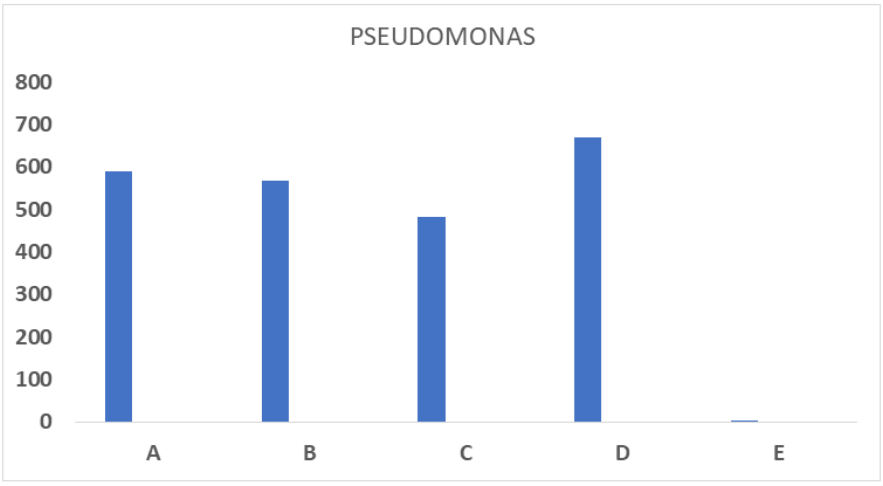

Fig 12: Average colony count of *Pseudomonas*in different experimental sets. A: Control, B: only extract, C: Only sunlight, D: Extract and UV ray, E: Extract and sunlight

Table 7: Colony counts of *Pseudomonas* spp. in different experimental sets

PSEUDOMONAS	Control	Only extract	Only sunlight	Extract+ UV ray	Extract+ sunlight
Colony count 1	596	588	482	686	2
Colony count 2	589	575	479	656	4
Colony count 3	590	544	485	669	5
Means±SD+ SEM	591.67±3.79±2.19	569±22.61±13.05	482±3±1.73	670.33±15.04±8.69	3.67±1.53±0.88

Table 8: P value matrix (*Pseudomonas* spp.) between different experimental sets (MedCalc statistical software)

	Control	Only extract	Only sunlight	Extract +UV ray	Extract + Sunlight
Control	-				
Only extract	0.0010	-	-	-	-
Only sunlight	<0.0001	0.0027	-	-	-
Extract +UV ray	0.0009	0.0030	<0.0001	-	-
Extract + Sunlight	<0.0001	<0.0001	<0.0001	<0.0001	-

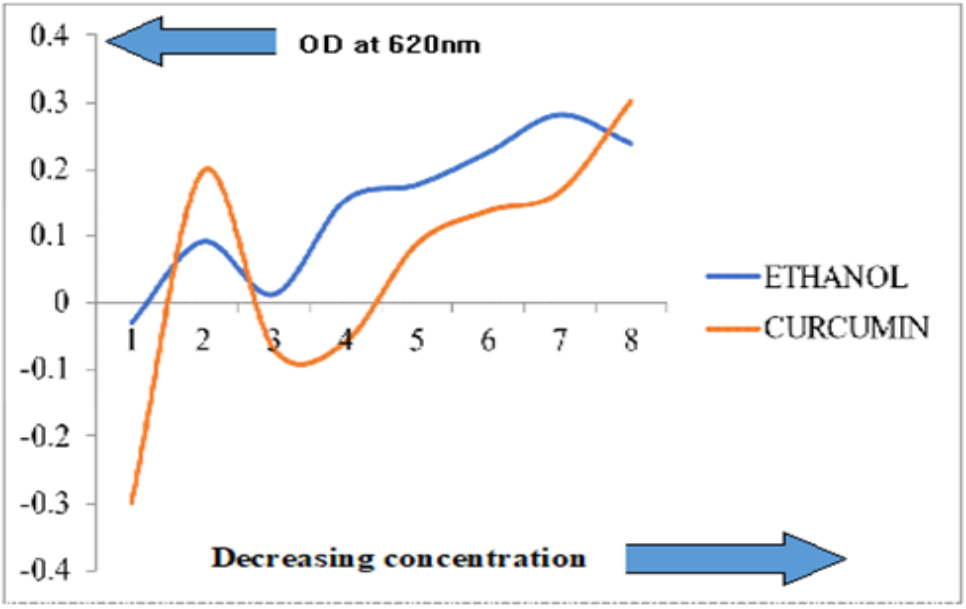

Fig 13: Showing effect of curcumin on the growth of MSSA (MIC value is 12.5mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml 4-6.125mg/ml, 5-3.125mg/ml, 6- 1.5625mg/ml, 7-0.78125mg/ml 8-0.390625mg/ml8-0.390625mg/ml

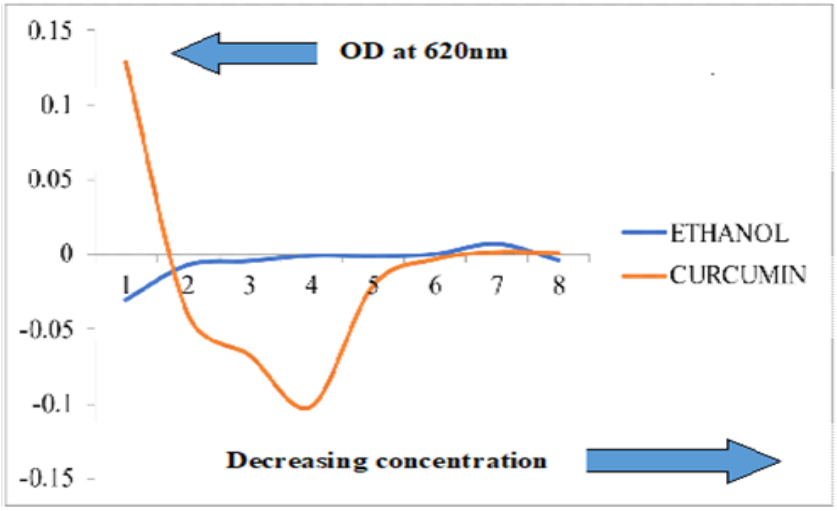

Fig 14: Showing effect of curcumin on the growth of MRSA (MIC value is 6.125mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5- 3.125mg/ml, 6-1.5625mg/ml, 7- 0.78125mg/ml

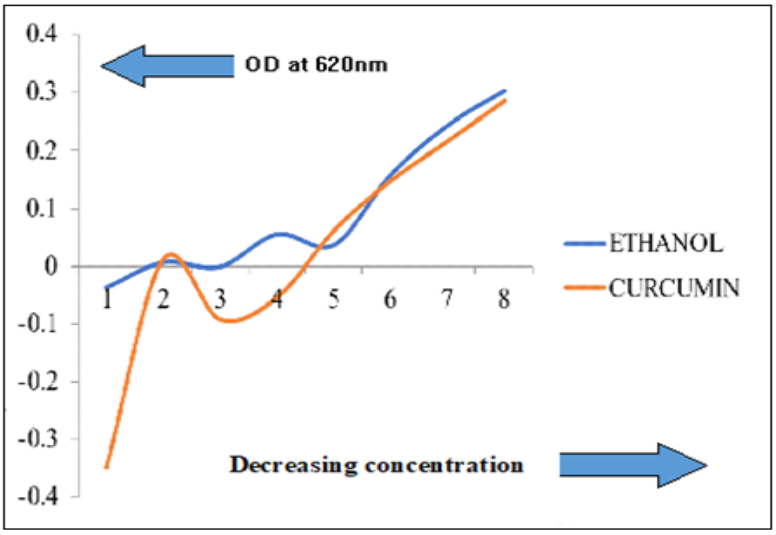

Fig 15: Showing effect of curcumin on the growth of *E.coli* (MIC value is 12.5mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml

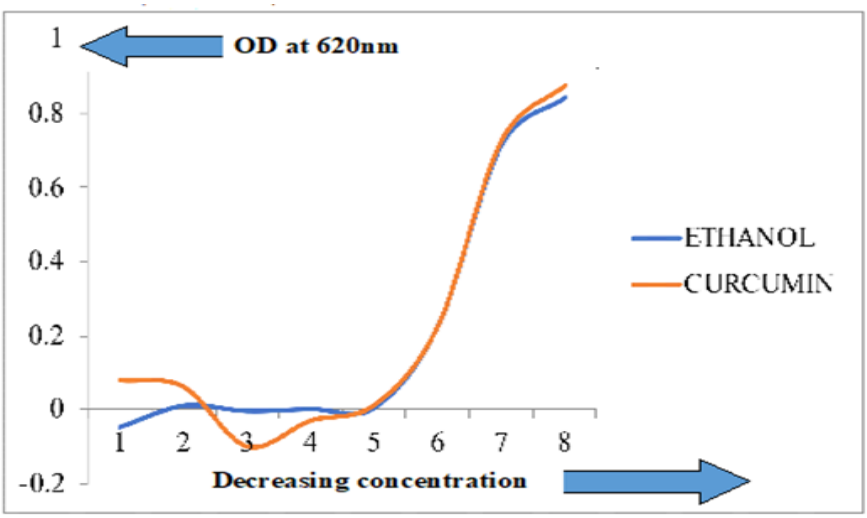

Fig 16: Showing effect of curcumin *Pseudomonas* spp. (MIC value is 12.5mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml

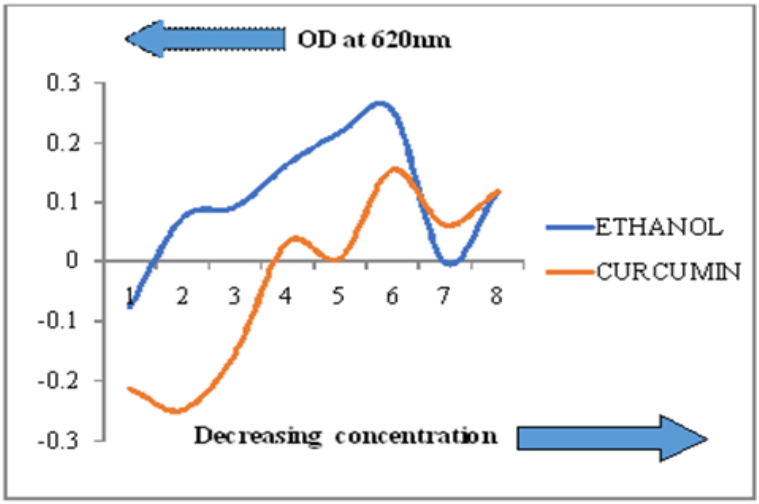

Fig 17: Showing effect of curcumin and sunlight on the growth of MSSA (MIC value is 1.5625mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.25mg/ml, 5-3.125mg/ml, 6-1.5625mg/ml, 7-0.78125mg/ml, 8- 0.390625mg/ml

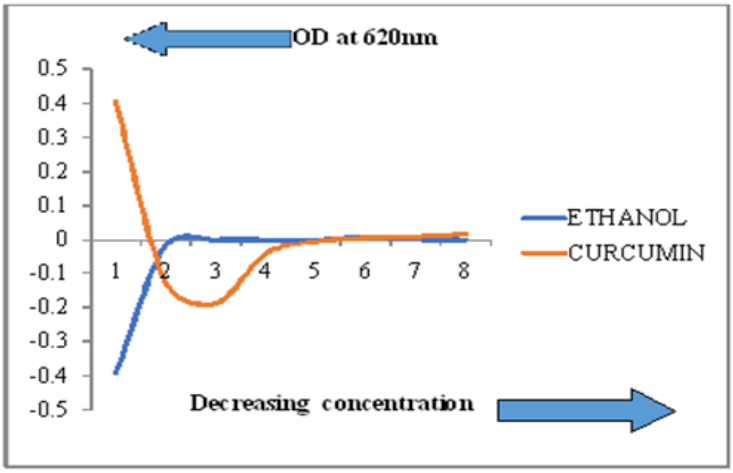

Fig 18: Showing effect of curcumin and sunlight on the growth of MRSA (MIC value is 12.5mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.25mg/ml, 5-3.125mg/ml, 6-1.5625mg/ml, 7-0.78125mg/ml, 8- 0.390625mg/ml

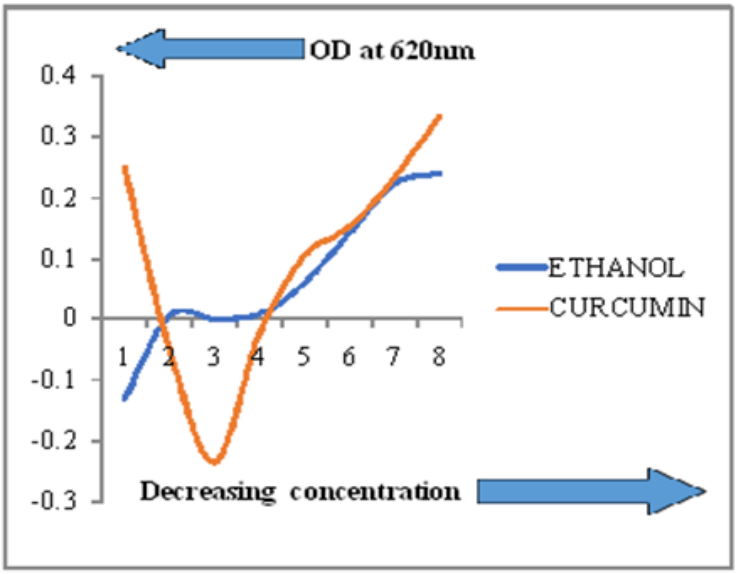

Fig 19: Showing effect of curcumin and sunlight on the growth of *E.coli* (MIC value is 25mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.25mg/ml, 5-3.125mg/ml, 6-1.5625mg/ml, 7-0.78125mg/ml, 8- 0.390625mg/m

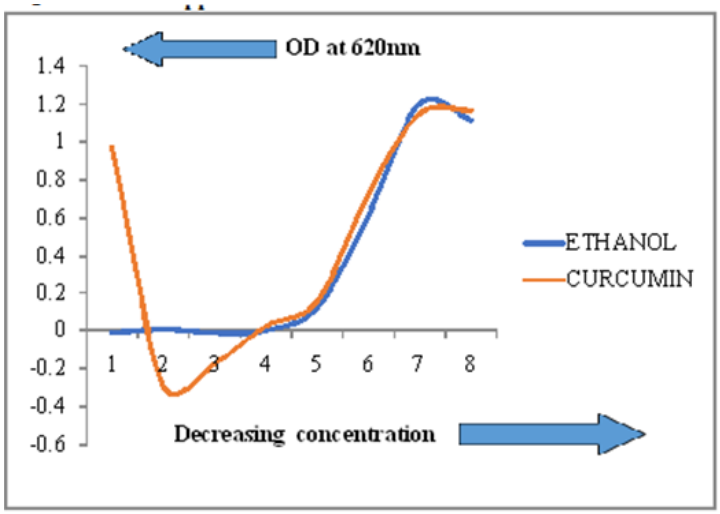

Fig 20: Showing effect of curcumin and sunlight on the growth of *Pseudomonas* spp (MIC value is 25mg/ml) 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml,4-6.25mg/ml, 5-3.125mg/ml, 6-1.5625mg/ml, 7-0.78125mg/ml, 8- 0.390625mg/m

DISCUSSION

The photodynamic mechanism is now widely used to eradicate cells showing rapid multiplication including bacterial cells. *Curcuma longa* contains nontoxic photosensitizer dye curcumin which can be activated by sunlight to produce reactive oxygen species inducing fatal damage to bacterial cells [5-7]. Although this mechanism is at present commonly used for the treatment of cancer cells, our study indicates that this can also be applied to stubborn bacterial infections. At present, this is termed photodynamic antimicrobial chemotherapy (PACT) [8, 9]. Initial observations by us indicate this can be used to treat surface infections and biofilms, looking forward to nano-targetted PACT development for the treatment of systemic infections.

Curcumin appears to be an effective natural photosensitizer. Recently we have studied a wide range of plants with numerous active components which can kill a wide spectrum of bacteria and fungi including MDR even pan-resistant microorganisms [10-16]. Curcumin can induce FtsZ assembly [17], and it can inhibit quorum sensing and cytokinesis [18]. MIC values showed a higher range which may be difficult for in vivo applications. Thus in these conditions, if a source of inducing photosensitizing activity of curcumin is added then it may act effectively in vivo.

UV light monotherapy is well known to be mildly effective on bacteria. When curcumin and sunlight both applied the action was excellent. This is probably due to UV-A of sunlight which is more damaging along with the heat of the sunrays. Thus further study on this line is essential.

CONCLUSION

Curcuma longa extract in sunlight shows excellent antibacterial action against both gram-positive and gram-negative bacteria. However, when a UV ray is used with curcumin the antibacterial action is mild. Thus at present curcumin with sunlight should be used for the treatment of MDR bacteria-induced surface infections until in vivo techniques are standardized for systemic MDR bacterial infections.

Conflict of Interest: The authors declare no conflict of interest.

Author's Contribution: Dr. Satadal Das designed the study procedure, analysed the data and corrected the manuscript. *Ms* Kayonika Bhadra and, Ms Ananya Basu performed the experiment under guidance of Mr. Arup Kumar Dawn, Dr. Bhaskar Narayan Chaudhuri and Dr. Partha Guchhait analysed the data and corrected the manuscript.

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