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Reduction of *Salmonella enterica* & *Staphylococcus aureus* Biofilm Development on Glass Tube by Plant Extracts

Md. Sultan Mahmud [a], Md. Ashiqur Rahman [b*], Md. Nazmul Islam [a], Sadia Islam [c], Sajib Kumar Saha [a], Md. Taijul Islam [a], Anny Khatun [a] and Silva Adhikari [a]

[a] Department of Microbiology, Jashore University of Science and Technology (JUST), Bangladesh.
[b] Department of Diagnostic, Novus Clinical Research Services Limited (NCRSL), Bangladesh.
[c] Department of Pharmacology, Bangladesh University of Health Sciences (BUHS), Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the present study is to evaluate anti-biofilm effect of the water soluble plant extracts such *Coccinia grandis, Terminalia arjuna, Centella asiatica against Salmonella enterica* and *Staphylococcus aureus* biofilm. Crude water soluble extracts of respective plants with different concentration was evaluated against biofilm adopting glass tube. Then washed with crystal violet dye and PBS buffer for observing the ring formation. Biofilm inhibition study revealed water soluble plant extracts inhibited biofilm formation. In our experiment we found that the plant extracts

**Corresponding author: E-mail: ararashiqur@gmail.com;*

Coccinia grandis, Terminalia arjuna & *Centella asiatica* gave excellent result for the reduction of biofilm of *Salmonella enteric & Staphylococcus aureus.* Water soluble *Coccinia grandis* extract is very effective for biofilm reduction than alcoholic *C. grandis* extracts. We measured the antiplantonic effect of these extracts by including the extracts into the nutrient agar media that containing respective organism by creating the hole into the plate then observed the result after 24-hour incubation. We also measured minimum inhibition concentration of these extracts through spectrophotometer with the help of the nutrient broth media. Bacterial motality was tested in petri plates with semi-solid medium (LB+0.4% agar) containing plant extracts and culture were inoculated in the center of the plate. This research will be very beneficial for us to reduce the pathogenic *S, aureus & S. enterica* biofilm by natural source especially plant extracts.

Keywords: Biofilm; Salmonella enterica; Staphylococcus aureus; plant extracts.

1. INTRODUCTION

In the field of medical microbiology, the emergence of infections that are resistant to treatment has been an urgent concern. Finding new antimicrobials is required because hospital illnesses caused by multidrug resistant microbiological diseases. The additional expense for treating the drug-resistant variant is about $10 billion a year in the US alone [1]. Compared to their planktonic counterparts, bacteria in biofilm are significantly more challenging to eradicate. Antimicrobials are known to be far more tolerant of and resistant to microbial biofilms than they are to the planktonic form of the same species. Cells attached to biofilms can increase their resistance to the actions of antimicrobial agents by a factor of 10 to 1000 [2]. Biofilms can be found in both biotic and abiotic surfaces [3]. The role of the biofilm is to attach to several solid surfaces, the epithelia of multicellular organisms and interfaces such as that between air and water [4]. In order for bacteria to arrange themselves effectively in their environment, surface adherence is an essential phase [5]. These microbial collectives are found to be ubiquitous in almost every environment [6]. On liquid surfaces, biofilms have been seen present as a floating mat and in a submerged state as well [7]. Either homogeneous or heterogeneous bacterial communities are present in biofilms, and they are embedded in an EPS matrix. Polysaccharides compose the majority of EPS, but it also contains proteins, lipids, and nucleic acids [3]. Polymers like glycopeptides, lipids and lipopolysaccharides form a scaffold and hold the biofilm together [8]. Biofilms have been found to technically be hydrogels due to the analysis of the EPS coat present in the biofilm, which reveals viscoelastic activity [9]. The biofilms can tolerate mechanical stress due to their characteristics. For the benefit of the bacterium, the nutrients included in the EPS matrix are trapped. By hydrogen bonding with the hydrophilic polysaccharides in EPS, the water that is already present in the matrix is also efficiently bound [10]. There have been reports of some bacterial biofilms having beneficial effects on food chains, sewage treatment facilities, and the prevention of petroleum oil and hydrocarbon spills into the oceans [11]. In nature, bacteria normally exist in groups termed biofilms that are connected to solid surfaces. Arthur Henrici first noted that the majority of aquatic microorganisms were aggregated over solid submerged surfaces rather than individual cells moving freely in 1933 [12]. The significance of biofilm development as a microbial survival strategy, however, as well as its huge effect on many human activities, have only recently come to light [13]. Since these populations are more resistant to the effects of antibiotics, the ability of bacteria to colonize solid surfaces in the biofilm form is a severe issue for both human and animal health (MAHTF). The development of pharmaceuticals is now thought to be primarily focused on the biofilm. By avoiding both host immune responses and antibiotic treatment, a biofilm helps bacteria persist [14].

According to the relative impermeability of biofilms, the varying physiological status of the microorganisms, the presence of subpopulations of persistent strains, and the variability in morphologies, biofilms render antibiotics useless [15]. Biofilms have been reported to show increased resistance to antimicrobial agents including antibiotics compared to free-floating cells [16]. Moreover, the use of synthetic drugs against the biofilm that are biochemically and genetically modified as a treatment are not reliable due to many controversial issues. Synthetic drugs may not be expensive but poses issues with adulterations and side effect. the action of these synthetic drug may be limited by their penetration and chemical reaction into

biofilm matrix, the extracellular polymeric material. Therefore, we need a new series of antimicrobial compounds that have a high efficiency and low cost. As a result, we have started to test natural products based materials such as edible medicinal plants. The biodiversity of plants provides an important source of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal, anti-cancer and antibiofilm activities [17]. In this regard, there is growing interest in plant extracts and other physiologically active chemicals derived from plants because they have long been used to treat disease and illness [18]. Modern scientific and technical developments are hastening the discovery and creation of novel medications with enhanced therapeutic activity and diminished plant-based negative effects. Due to their reputation as being safe and their long history of usage in folk medicine as immune boosters and for the prevention and treatment of many ailments, plant chemicals are generally acknowledged [19]. Over time, the usage of medicinal plants—the foundation of traditional medicine—has increased, with an estimated 80% of population, especially in poorer nations, turning to them for their primary healthcare [20]. Crude extracts of leaves, roots, and stems, as well as specific chemicals extracted from these, as well as essential oils and essential oil constituents, are among the plant-derived substances undergoing significant research for potential applications in the pharmaceutical sector. Although there is now a lot of study on plants and their active ingredients, the main emphasis is on the antibacterial activities against planktonic or bacteria that form biofilms [21].

A Gram-negative bacterium called Salmonella enteric causes four distinct clinical manifestations: enteric fever, bacteremia, gastroenteritis, and an asymptomatic carrier state [22]. It is more common in children under the age of 5, adults 20-30 year olds and patients 70 years or older [22]. *Staphylococcus aureus* is an opportunistic pathogen that can cause a variety of self-limiting to life-threating diseases in humans [23].The bacteria are a leading cause of food poisoning, resulting from the consumption of food contaminated with enterotoxins [24]. Staphylococcal food intoxication involves rapid onset of nausea, vomiting, abdominal pain, cramps and diarrhea [23]. Bangladesh has very rich in biodiversity. It has more than 500 medicinal plants species [25]. *Coccinia grandis*, *Terminalia arjuna* & *Centella asiatica* are the most common medicinal plant in Bangladesh. The leaf extract of *Coccinia grandis* & *Centella asiatica* and the bark of *Terminalia arjuna* have many therapeutic activities. In the present study, anti-biofilm effect of *Coccinia grandis, Terminalia arjuna, Centella asiatica* against *Salmonella enterica* and *Staphylococcus aureus* biofilms was discussed.

2. METHODOLOY

2.1 Stock Cultural Preparation

A stock culture is “a culture of a microorganism maintained solely to keep it viable for subculture into fresh medium”. A working culture is defined as “a microorganism preparation derived from a reference stock culture used as a control on a regular day to day basis”. First we revive the pure culture of *Staphylococcus aureus* and *Salmonella enterica* on selective media (mannitol salt agar and *Salmonella shigella*-agar) and incubated at 37°C for 24 hrs. *S. aureus* give yellow colonies and *S. enterica* give black colonies. Then these single colonies are inoculated separately in 7 ml nutrient broth within each test tube and incubated at 37°C for 24 hrs. It is also called enrichment of organism. 1.5 ml enriched culture were taken in autoclaved Eppendorf tube. Then Eppendorf tubes were centrifuged at 1200 rpm for 7 min. After centrifugation supernatant was separated from the pellet and then removed it from Eppendorf tube. Autoclaved glycerol broth (50% glyserine+50% distilled water) were added in each pellet containing Eppendorf tube and stored at -20°c for long term used.

2.2 Bacteria and Culture Medium

We used two different bacteria to evaluate the extracts' capability to prevent biofilms:

***Staphylococcus aureus*:** It is an important laboratory strain; it has medical interest because it causes several skin infections.

***Salmonella enterica*:** It is an important laboratory strain; this species is of medical interest because they cause several intestinal illnesses. Cultures (*S.aureus* and *S.enterica*) were grown at 37°C in nutrient broth media.

2.3 Plant Extracts Preparation

Certain plant materials are not identified in the table for results protection reasons.

Table 1. Plants used in this work are summarized in below table [16,18]

Extract number	Common name	Scientific name	Materials	Known attributes or traditional medicine uses
1	Neem	*Azadiracta indica*	Leafs	Skin ulcers, diabetes
2	Gritkumari	*Aloe verra*	Leaves	Constipation, diabetes
3	Telakochu	*Coccinia grandis*	Leaves	Kidney stones, fatigue
4	Pathorkuchi	*Kalanchoe pinnata*	Leaves	Intestinal problems, dysentery
5	Thankuni	*Centella asiatica*	Leaves	Anxiety, hypertension, arthritis
6	Ulotkombol	*Abroma Augusta*	Leaves	Gonorrhea, diabetes, headaches with sinusitis.
7	Arjun	*Terminalia arjuna*	Bark	Chronic fever, Sinusitis, cough, urine retention
8	Durbaghas	*Cynodon dactylon*	Whole plant	Prickly heat, digestive disorder, diabetes.

We collected most plant material (leaves, bark) from different sides of Jessore University of Science and Technology (JUST). Besides, we included other plants according to their medical attributes, based on information found on the internet. To obtain the extracts, at first we washed the plant material with tap water, grinded with sterile mortar and adding distilled water gradually. By squeezing plant extract, we reserve it in glass bottle. Then filtered with syringe filter and stored in glass tube.

2.4 Biofilm Assays

Biofilm formation was studied in glass test tubes. In each test tube 5 ml of nutrient broth medium was inoculated with 20 µl of an overnight culture of the chosen bacterium and we added 100µl of extract. As a control, the same volumes of overnight culture were used. Test tubes were incubated at 37°C for 24 hours and then the biofilms stained 2% crystal violet dye.

2.5 Staining Techniques

Another method used in microscopy to improve contrast in the microscopic image is staining. We utilized a 2% solution of crystal violet, a dye that colors bacteria's polysaccharides, to see and measure biofilms. Each well or tube received 1.5 ml or 5 ml of crystal violet, accordingly. The dye was removed after the solution had rested for 10 minutes. PBS buffer was used to wash the tubes twice, and then they were given time to dry. The amount of bacterial biomass adhered to the surface is indicated by the intensity of the violet color that still appears on the tube. To obtain quantitative data, the dye was solubilized with 70% ethanol and color intensity was measured in a spectrophotometer as absorbance at a wavelength of 540 nm. Biofilm reduction concentration were measured by using following equation:

- Biofilm inhibition/eradication (%) = [1 − (OD 540 nm of test compound) / (OD 540 nm of control)] × 100%.

2.6 Agar Well Diffusion Method

The antibacterial activity of plant extracts is assessed using the agar well diffusion method. A volume of the microbial inoculum is dispersed over the entire agar surface, much as the process employed in the disk diffusion method, to inoculate the agar plate surface. Then, a (100 µl) volume of the microbial extract solution at the specified concentration is added to the well by aseptically punching a hole with a diameter of 6 to 8 mm. Agar plates are then incubated at 37^0C, and the outcome is then observed.

2.7 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) in microbiology is the lowest concentration of a substance that stops a bacterium from growing visibly. By creating solutions of the chemical at escalating concentrations, the MIC of a substance is ascertained. At first nutrient broth media were prepared. After autoclaving, 20 µl culture of *S.enterica, S.aureus* and 100 µl plant extracts were added into 5ml nutrient broth containing test-tubes. Then the tubes were incubated at 37°C for 24 hours. After incubation, planktonic cells concentrations were measured at

540 nm by spectrophotometer using following equation:

- Biofilm inhibition/eradication (%) = [1 − (OD 540 nm of test compound) / (OD 540 nm of control)] × 100%.

2.8 Swimming Motility

Motility in biology is the capacity to move actively and spontaneously while expending energy in the process. In Petri plates with semi-solid medium (LB+0.4%agar) containing plant extract and media without plant extract were used as controls, bacterial motility was examined. The center of the plate was injected with 2 l of a growing culture, and the plates were then incubated for 24 hours at 37°C. The image of a swimming halo was seen. We started out with 0.5% agar. Media had been fixed and motility findings were not visible while applying this concentration. Then we applied 0.4% agar, which effectively displayed the outcome.

3. RESULTS

3.1 Biofilm Assays

First we tested the effect of adding *Cocciniagrandis, Terminaliaarjuna* and *Centellaasistica*, *C.dactylon, Aloe verra, Kalanchoepinnata, Abromaaugustum, Azdiractaindica*plant extracts on *S.enterica and S.aureus* biofilm formation in glass tubes (100 µl of extract in 5 ml). Bacterial cultures were observed after 24-hour incubation and then stained with2% crystal violet as described in Materials and Methods. *S.enterica and S.aureus* normally forms a ring on the control tube surface. Fig. 1(a) and 1(b) displays the outcomes from some extracts.

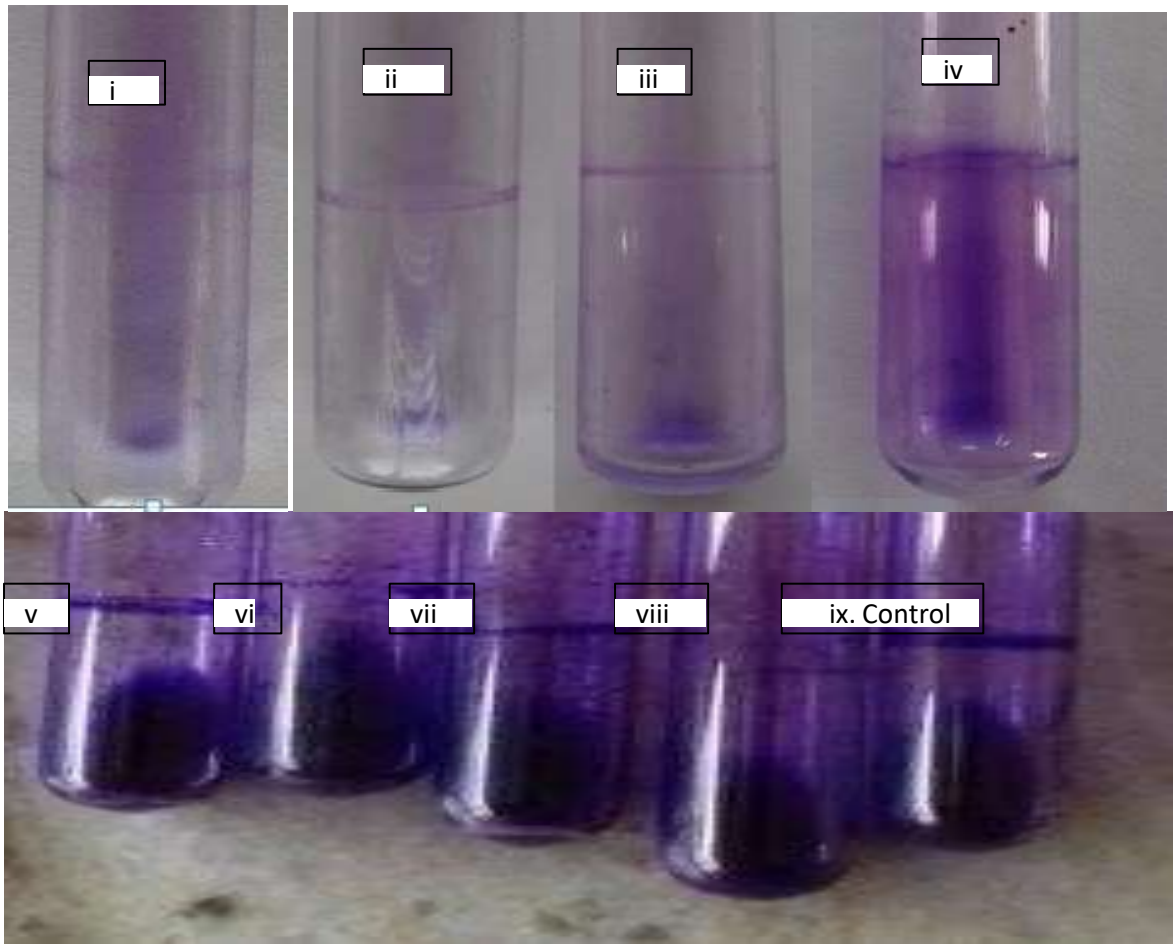

Fig. 1(a). Formation *of S.enterica* biofilm in the presence *of plant extracts: (i) T.arjuna,(ii) C.grandis (iii)C.asiatica (iv)C.dactylon (v) Aloe verra (vi)Kalanchoepinnata (vii)Abromaaugustum (viii)Azdiractaindica (ix) Control(no extracts)*

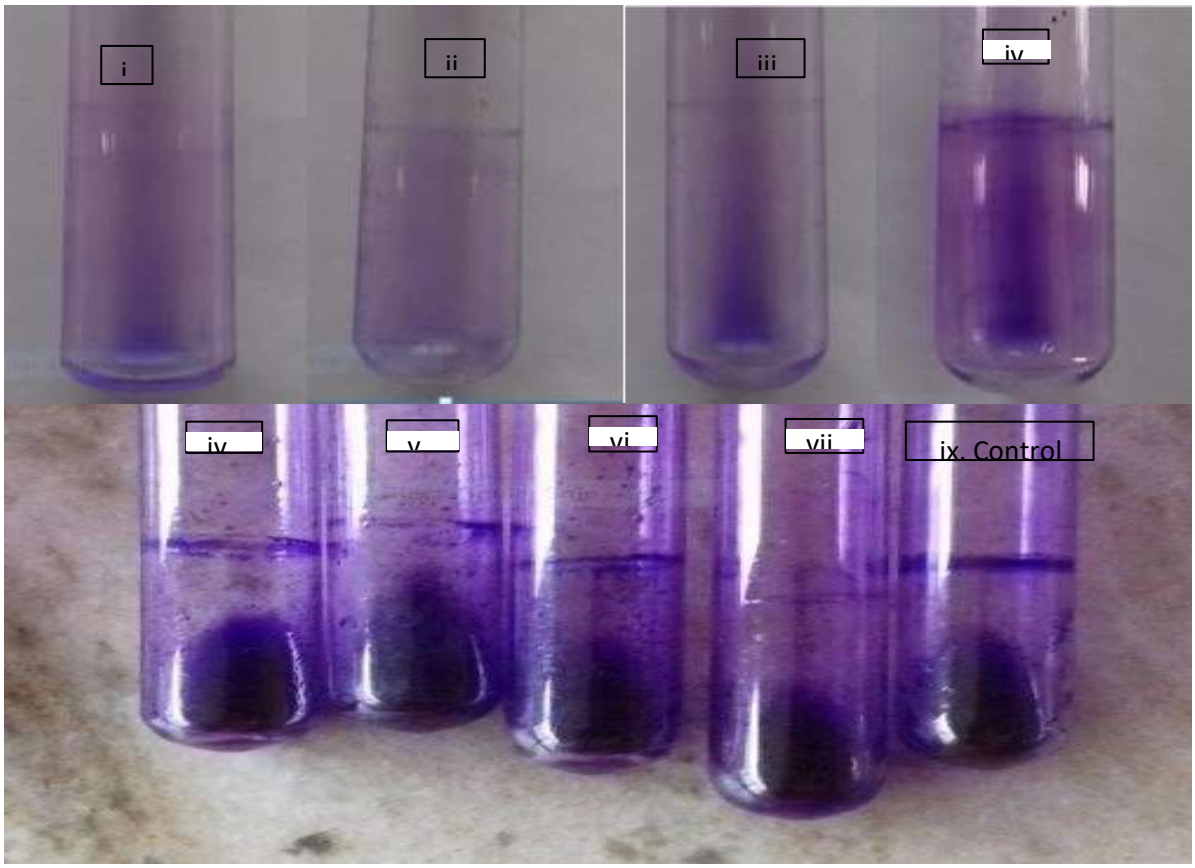

Fig. 1(b). *S.aureus* biofilm formation in the presence of plant extracts:(i*) T.arjuna* (ii) *C.grandis* (iii) *C.asiatica* (iv)*C.dactylon (v)Aloe verra (vi)Kalanchoe pinnata (vii)Abroma augustum (viii) Azdiracta indica (ix)* Control (no extracts)

After staining, more visible color was observed in *Salmonella enterica* and *Staphylococcus aureus* control tubes. In the case of *S.enterica*, color intensity of the Arjun contain tube was less clear than the other extracts containing tubes. After staining, with extract C. grandis and C. asiatica some of the biofilm has shed. With extracts *C. dactylon, Aloe verra, Kalanchoe pinnata, Abroma augustum, Azadiracta indica* we observed spread of biofilm. There is less staining with extract *T. arjuna* than in the control. Also in the case of *S. aureus* color intensity of the *Coccinia grandis* containing tube was less clear than the other extracts containing tube. After staining, with extract *T. arjuna* and *C. asiatica* some of the biofilm has shed. With extracts *C. dactylon, Aloe verra, Kalanchoe pinnata, Abroma augustum, Azadiracta indica* we observed spread of biofilm. There is less staining with extract *C. grandis* than in the control.

3.2 Antibiofilm Potential of Plant Extracts

In this study, anti-biofilm (biofilm reduction) potential of *T. arjuna, C.grandis and C. asiatica* plant extracts was also evaluated and other extracts are not evaluated because they have no effect for biofilm reduction assay. Extracts of *Terminalia arjuna* is played most potent S. enterica biofilm reduction (91.75%) and extracts of *Coccinia grandis* and *Centella asiatica* also reduced 89.54% and 80.21% biofilm respectively.

Also extracts of *Coccinia grandis* displayed most potent *S. enterica* biofilm reduction (86.88%) and extracts of *Terminali aarjuna* and *Centella asiatica* also reduced (80.97% and 82.27%) biofilm respectively and other extracts are not used because they have no effect for this biofilm reduction assay.

3.3 Minimum Inhibition Concentration (MIC)

It was found that the MIC of *Centella asiatca*, *Terminalia arjuna, Coccinia grandis* were 2.27%, 1.60%, 1.13% for the Salmonella enterica.

Table 2. Reduction of *Salmonella enterica* biofilm on define plant extracts

Name of the organism	Name of the extracts	Amount of culture	Amount of extracts	Optical density	%BIC
Salmonella enteric	Control (no extract)	20 µl	0	2.40	0.00
Salmonella enteric	*Coccinia grandis*	20 µl	100 µl	0.251	89.542
Salmonella enterica	*Terminalia arjuna*	20 µl	100 µl	0.198	91.75
Salmonella enterica	*Centella asiatica*	20 µl	100 µl	0.475	80.208

Table 3. Reduction of *Staphylococcus aureus* biofilm on define plant extracts

Name of the organism	Name of the extracts	Amount of culture	Amount of extracts	Optical density	%BIC
Staphylococcus aureus	Control(no extract)	20 µl	0	1.76	0.00
Staphylococcus aureus	*Coccinia grandis*	20 µl	100 µl	0.231	86.875
Staphylococcus aureus	*Terminalia arjuna*	20 µl	100 µl	0.335	80.966
Staphylococcus aureus	*Centella asiatica*	20 µl	100 µl	0.312	82.273

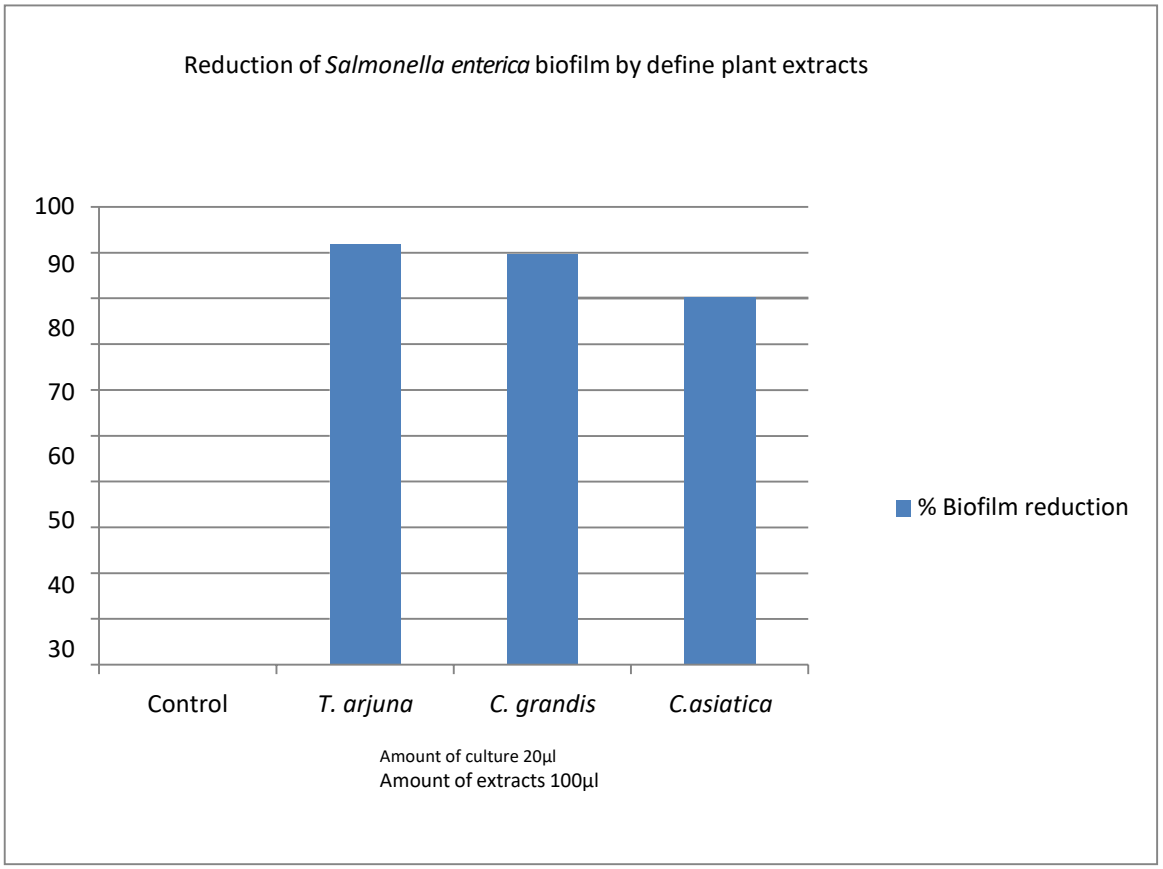

Fig. 2(a). Graphical representation of reduction *S.enterica* biofilm on define plant extracts

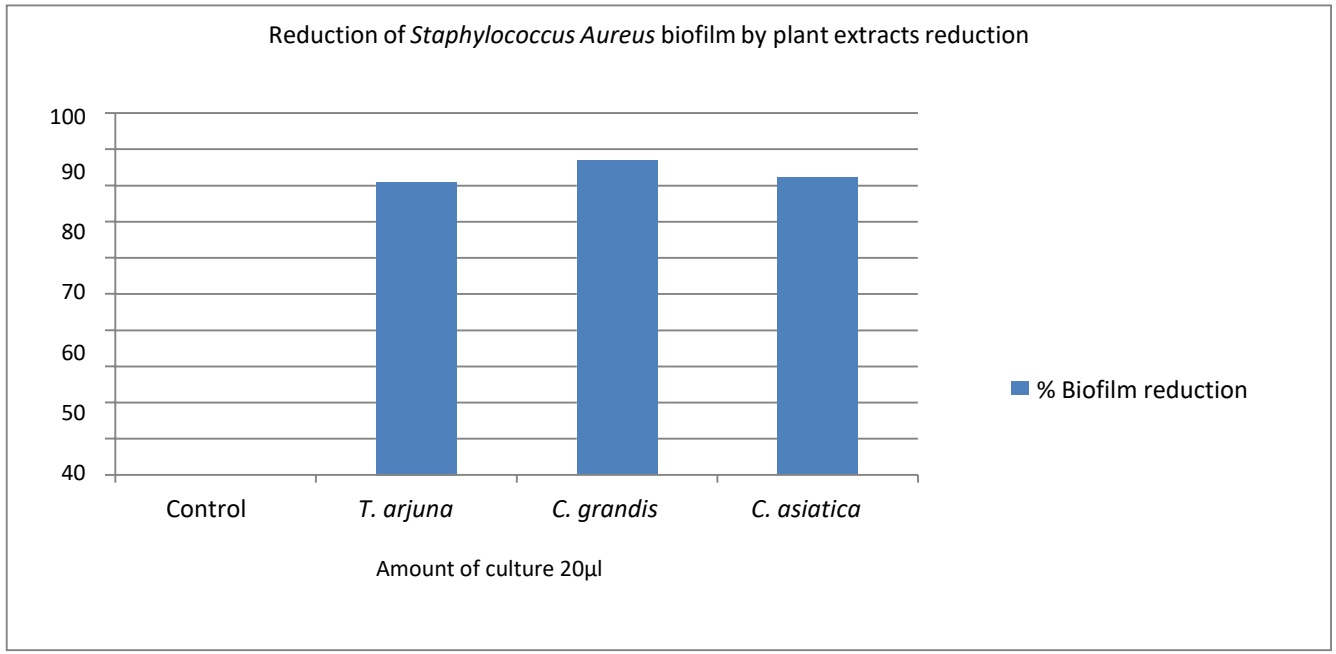

Fig. 2(b). Graphical representation of reduction *S. aureus* biofilm on define plant extracts

Table 4(a). Minimum inhibition concentration of *Salmonella enterica* on define plant extracts

Name of the organism	Name of the extracts	Amount of culture	Amount of extracts	Optical density	% MIC
Salmonella enteric	Control (no extract)	20 µl	0	4.40	0
Salmonella enteric	*Coccinia grandis*	20 µl	100 µl	4.35	1.13
Salmonella enteric	*Terminalia arjuna*	20 µl	100 µl	4.33	1.60
Salmonella enteric	*Centella asiatica*	20 µl	100 µl	4.30	2.27

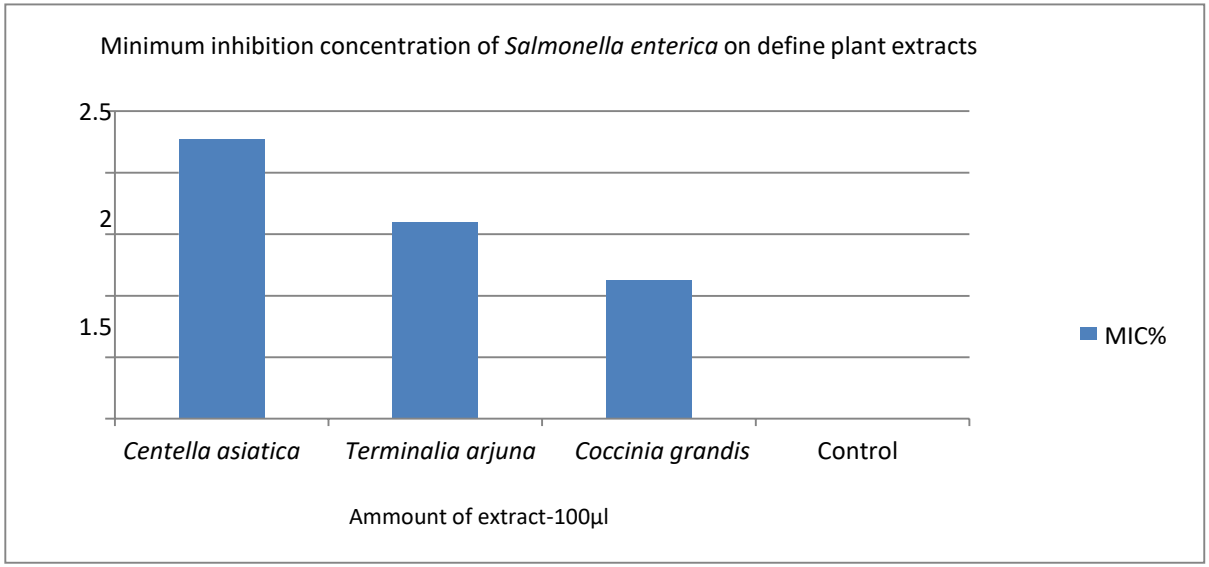

Fig. 3(a). Graphical representation of MIC *S. enterica* on define plant extracts

Table 4(b). Minimum inhibition concentration of *Staphylococcus aureus* on define plant extracts

Name of the organism	Name of the extracts	Amount of culture	Amount of extracts	Optical density	%MIC
Staphylococcus aureus	Control (no extract)	20 µl	0	4.43	0
Staphylococcus aureus	*Coccinia grandis*	20 µl	100 µl	4.30	2.93
Staphylococcus aureus	*Terminalia arjuna*	20 µl	100 µl	4.37	1.35
Staphylococcus aureus	*Centella asiatica*	20 µl	100 µl	4.32	2.48

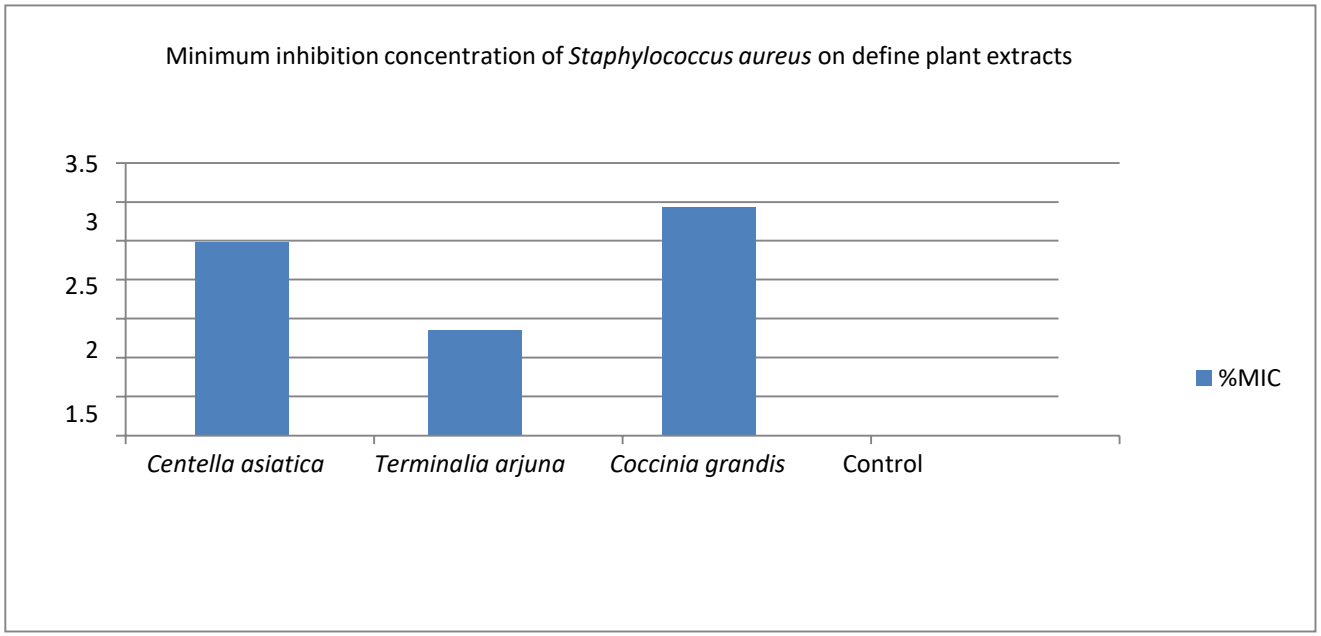

Fig. 3(b). Graphical representation of MIC of *S.aureus* on define plant extracts

It was found that the MIC of *Centella asiatca, Terminalia arjuna, Coccinia grandis* were 2.48%, 1.35%, 2.93% for the *Staphylococcus aureus*.

3.4 Antiplantonic Effect

Our desired plant extracts have no antiplantonic effect against the test organism. For this reason, there is no zone of inhibition were found in the petri plate by using agar well diffusion method. Show in Fig. 4.

3.5 Swimming Motality

We tested if our desire plant extracts altered motility of *S. aureus* and *S. entericain* plates with a semi-solid medium (LB + agar 0.4%), where bacteria can swim, forming a large halo in control plate from the point of inoculation. Results are presented in fig. 5 (a).

(i) **(ii)**

Fig. 4. Antiplantonic effect of define plant extracts against (i) *S. enterica (*ii) *S.aureus*

Fig. 5(a). Swimming motility of S. enterica in the presence of plant extracts. (i) control (no extracts) (ii) *T. arjuna* (iii) *C. grandis* (iv) *C. asiatica*

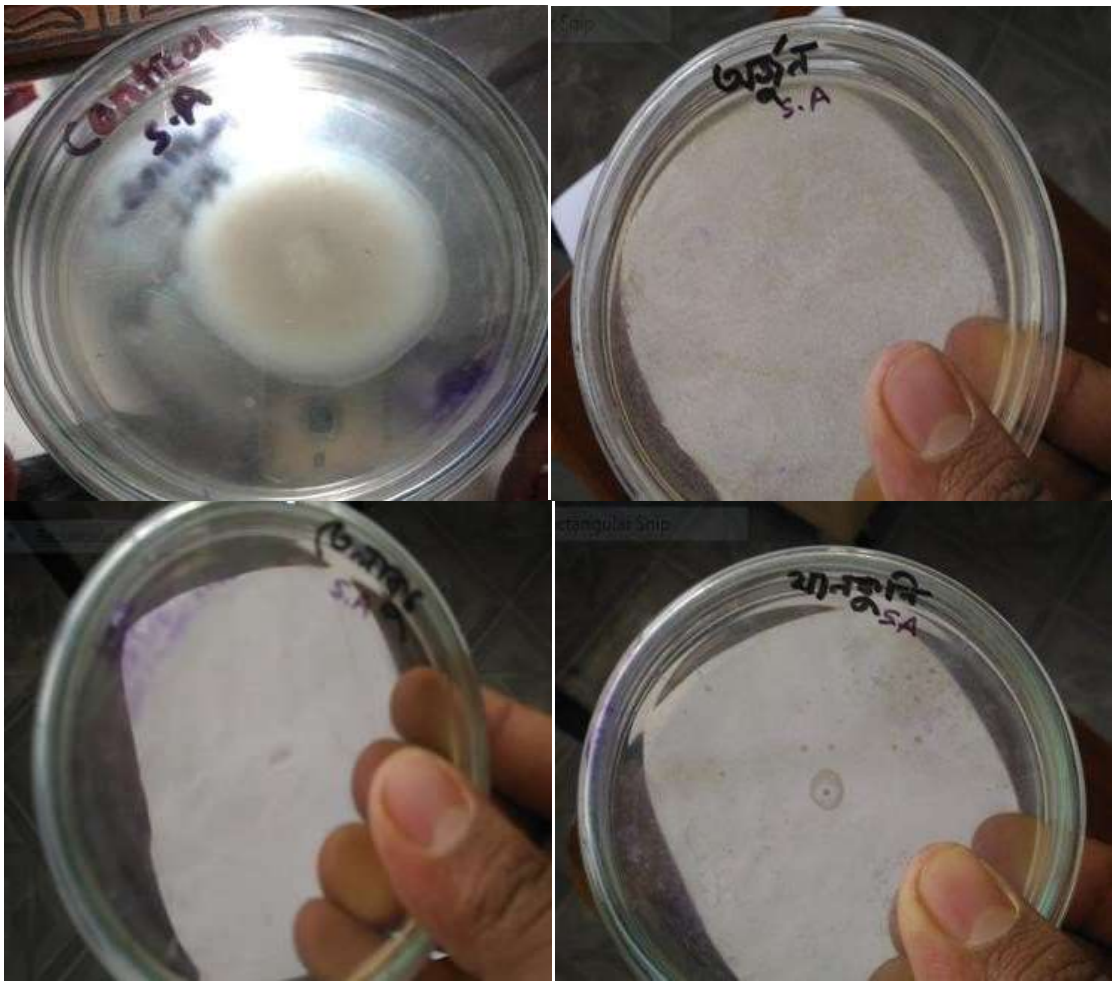

Fig. 5(b). Swimming motility of *S. aureus* in the presence of plant extracts. (i) Control plate (no extract) (ii) *T. arjuna* (iii) *C. grandis* (iv) *C. asiatica*

4. DISCUSSION

The past few years have seen the isolation of numerous natural anti-microbial agents from a variety of bacterial, plant, and animal species. A significant and easily accessible resource for primary care and complementary care systems is herbal medicine. The pathogens are developing resistance to the existing antibiotics, thus they may be the best substitute. They have no side effects like commercial drugs. Bacteria are more resilient to different antimicrobial treatments when they are in the biofilm state. These substances, which make up a wide class of chemicals employed in natural host defense, may be utilized to treat human infections and are currently thought to be alternate forms of therapy. In clinical and industrial contexts, bacterial biofilm formation can result in major issues, which has led to the invention or testing of biofilm inhibitors. Bacteria are more resilient to different antimicrobial treatments when they are in the biofilm state. Researchers have been compelled to find alternate methods for treating infections because of the complexity of the majority of microbial illnesses increasing and the resistance to traditional medication. Since they have been used to treat illnesses and diseases for thousands of years, plant extracts and other biologically active chemicals that have been extracted from plants have attracted a lot of attention in this area. The current work used a biofilm inhibition spectrophotometric assay to examine the anti-biofilm activity of plant extracts against *Staphylococcus aureus* and Salmonella enterica. All of the investigated plant extracts reduced biofilm in a dose-dependent way.

Table 5(a). Diameter of swimming motility halo of *Salmonella enterica* in the presence of different extracts

Name of the Organism	Name of the Extract	Amount of Extract	Amount of Culture	Incubation Time (Hour)	Diameter of Halo(cm)
Salmonella enterica	Control (no extract)	0.00	One loop	16 h	3.2 cm
Salmonella enterica	*Coccinia grandis*	300 µl	One loop	16 h	0.00 cm
Salmonella enterica	*Terminalia arjuna*	300 µl	One loop	16 h	0.00 cm
Salmonella enterica	*Centella asiatica*	300 µl	One loop	16 h	0.50 cm

Table 5(b). Diameter of swimming motility halo of *Staphylococcus aureus* in the presence of different extracts

Name of the Organism	Name of the Extract	Amount of Extract	Amount of Culture	Incubation Time (Hour)	Diameter of Halo (cm)
Staphylococcus aureus	Control (no extract)	0.00	One loop	16 h	3.5 cm
Staphylococcus aureus	*Coccinia grandis*	300 µl	One loop	16 h	0.00 cm
Staphylococcus aureus	*Terminaliaarjuna*	300 µl	One loop	16 h	0.00 cm
Staphylococcus aureus	*Centella asiatica*	300 µl	One loop	16 h	0.30 cm

However, in our study the biochemical composition of the *Salmonella enterica* & *Staphylococcus aureus* biofilm matrix has been highly reduced by the watery plant extract *Coccinia grandis, Terminalia arjuna & Centella asiatica.* Out of these three plants *T. arjuna* is very effective for the *S. enterica* and *C. grandis* also for *S. aureus* microorganism. *T. arjuna reduces 91.75% S. enterica biofilm and other plants also reduces it above 80%*. *T. arjuna* bark is very effective for gastrointestinal diseases and *S. enterica* is mostly responsible for these disorder. The aqueous bark extracts of *T. arjuna* lacked antifungal activity against *C. albicans* and antibacterial activity against *S. aureus* that was found sensitive both to the hot and cold aqueous bark extracts [23]. So the extracts strongly fight with this organism and reduces its biofilm mostly. In the case of *S. aureus, C. grandis* is very effective and reduces 86.875% biofilm, other plants also reduce above 80% *S. aureus* biofilm. *S. aureus* is an opportunistic pathogen but in immune suppress patient they produce mainly skin disorder and *C. grandis* leaf paste is very useful for that disorder, So it is said that all plant extracts are very strongly reduces the biofilm of these pathogenic organisms. Peppermint essential oil has been shown to be effective against biofilm formation by *Salmonella enterica* and *Candida albicans* [26]. Our desired plant extracts have no anti-planktonic activity against test organisms. Our desired plant extracts have no anti-planktonic activity against test organisms. For that reason, planktonic cell concentration in the extract containing tubes are very similar with control tubes (no extract). *Centella asiatica* leaves (water soluble) showed best anti-microbial activities against Mycobacterium spp [27]. But they reduce the swimming motility of these organisms.

Swimming motility is the key factor for the biofilm formation without it, organisms are unable to produce biofilm in the surfaces [28]. All organisms produce biofilm except genetically modified organisms but without motility they are strongly unable to form it [29]. Delft University of Technology Both free and chitosan coated plant extracts inhibited biofilm formation by *E. coli*, and enhanced effect on biofilm inhibition was recorded in polymer coated extracts of the all the tested plants [30,31]. This result indicated that *T. arjuna, C. grandis and C. asiatica are* more effective to the test microorganisms. These plant leaves and bark may be indicated as beneficial sources to create natural bioactive compounds from which we could develop fresh, cost-effective antibiotics. In vivo models are required to examine the effects of the agent on health.

5. CONCLUSION

S. aureus causes gastro-enteritis food poisoning *S. enteric* are closely related to the *Escherichia* genus and are found worldwide causes illnesses in humans and many animals, such as typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis [32]. It can be concluded that *Coccinia grandis, Terminalia arjuna, Centella asiatica, Cynodon dctylon, Alovera, Abroma augustum, Azardirchata indica, Kalanchoe pinnata* is an important source of many pharmacological and medicinally important chemicals. The present study was carried out on a preliminary basis, in order to identify the plants that capable of reduce *S. enterica* and *S. aureus* biofilms. The extracts of *Coccinia grandis*, *Centella asiatica, Terminalia arjuna* were able to inhibit biofilm of *Staphylococcus aureus& Salmonella enterica* biofilm. By understanding the true mechanism of anti-biofilm effect of these extracts can help to fight against infections due to *Staphylococcus aureus* & *Salmonella enterica*. The use of these plant extracts as therapeutic agent could help to save cost and reduce chemical drug toxicities or side effects. Further experiments are required to study more and more extracts in detail, their potential as anti-biofilm effect on health and component analysis of these plant extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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