Original Research Article

Reduction of *Salmonella enterica & Staphylococcus aureus* biofilmdevelopmentonglass tubebyplantextracts

ABSTRACT

The objective of the present study is to evaluate anti-biofilm effect of the water soluble plantextracts such *Cocciniagrandis,Terminalia arjuna,Centellaasiatica against Salmonella enterica*and*Staphylococcusaureus*biofilm.Crudewatersolubleextractsofrespectiveplantswithdifferent concentration was evaluated against biofilm adopting glass tube.Then washed withcrystal violet dye and PBS buffer for observing the ring formation.Biofilm inhibition studyrevealed water soluble plantextractsinhibitedbiofilmformation. In our experiment we foundthat the plant extracts*Cocciniagrandis,Terminalia arjuna*&*Centellaasiatica*gave excellentresult for thereduction of biofilm of *Salmonella enteric & Staphylococcus aureus.* Watersoluble *Cocciniagrandis*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 thenutrient agar media that containing respective organism by creating the hole into the plate thenobserved the result after 24-hourincubation. We also measured minimuminhibition concentrationof these extracts through spectrophotometer with the help of the nutrient broth media.Bacterialmotality was tested in petri plates with semi-solid medium(LB+0.4% agar) containing plantextracts and culture were inoculated in the center of the plate. This research will be very beneficialfor us to reduce the pathogenic *S,aureus &S. enterica* biofilm by natural source especially plantextracts.

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]. Biofilmscanbefoundinbothbioticand abioticsurfaces[3] .The role of the biofilmis to attach to several solid surfaces, the epithelia of multicellular organisms and interfaces suchas 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 inalmosteveryenvironment[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] .Polymerslikeglycopeptides,lipidsandlipopolysaccharidesformascaffoldandholdthebiofilmtogether[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]. Biofilmshavebeenreportedtoshowincreasedresistance to antimicrobial agents including antibiotics compared to free-floatingcells[16].Moreover, the use of synthetic drugs against the biofilm that are biochemicallyand genetically modified as a treatment are not reliable due to many controversialissues.Synthetic drugs may not be expensive but poses issues with adulterations and side effect.the actionof these synthetic drugmay be limited by their penetration and chemical reaction intobiofilm matrix, the extracellular polymeric material. Therefore, we need a new series ofantimicrobialcompoundsthat haveahighefficiencyandlow cost.As a result, wehavestartedtotest natural products based materials such as edible medicinal plants. The biodiversity of plantsprovides an important source of chemical compounds,which have many therapeutic applicationssuch as antiviral,antibacterial,antifungal,anti-cancer and antibiofilmactivities[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 populations, 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 yearolds and patients 70 years or older[22]. Staphylococcus aureus is anopportunistic pathogen that can cause a variety of self-limiting to life-threating diseases inhumans[23].The bacteria are a leading cause of food poisoning, resultingfrom the consumption of food contaminated with enterotoxins[24].Staphylococcalfood intoxication involves rapid onset of nausea,vomiting,abdominalpain, cramps anddiarrhea[23].Bangladesh has very rich in biodiversity. It has more than 500medicinal plants species[25].*Cocciniagrandis,Terminalia arjuna*&*Centellaasiatica* are the most common medicinal plant in Bangladesh.The leaf extract of *Cocciniagrandis*&*Centellaasiatica* and the bark of Terminalia arjuna have many therapeutic activities.Inthe present study, anti-biofilm effect of *Cocciniagrandis,Terminalia arjuna,Centellaasiatica*against *Salmonella enterica*and *Staphylococcus aureus*biofilmswas discussed.

2. METHODOLOY

2.1 Stock Cultural Preparation

A stock culture is "a culture of a microorganism maintained solely to keep it viable forsubcultureintofreshmedium".Aworkingcultureisdefinedas"amicroorganismpreparationderivedfrom areferencestockcultureused asacontrol on aregulardaytodaybasis".First we revive the pure culture of *Staphylococcus aureus* and *Salmonella enterica* on selectivemedia (mannitol salt agar and Salmonella shigella-agar) and incubated at 37°C for 24 hrs.S.aureusgive yellow colonies and *S.enterica* give black colonies. Then these single colonies areinoculated separately in 7ml nutrient broth within each test tubeand incubated at 37°C for 24hrs. It is also called enrichment of organism.1.5ml enriched culture were taken in autoclavedEppendorf tube. Then Eppendorf tubes were centrifuged at 1200 rpm for 7 min. Aftercentrifugation supernatant was separated from the pellet and then removed it from Eppendorftube. Autoclaved glycerol broth (50% glyserine+50% distilled water) were added in each pelletcontainingEppendorftubeand storedat-20°cfor longtermused.

2.2 Bacteriaandculturemedium

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

2.2.1 ***Staphylococcusaureus*:**Itisanimportantlaboratorystrain;ithasmedicalinterestbecauseitcausess everal skin infections.

2.2.2 ***Salmonellaenterica*:**Itisanimportant laboratorystrain;thisspecies isofmedicalinterestbecausetheycause several intestinal illnesses.Cultures(*S.aureus*and*S.enterica*)weregrownat37°Cinnutrientbrothmedia.

2.3 Plantextractspreparation

Certainplantmaterialsarenotidentifiedinthetableforresultsprotectionreasons.

Table1: Plants used inthiswork aresummarized in belowtable[16,18]

Extract number	Commonname	Scientificname	Materials	Knownattributesortraditional medicineuses
1	Neem	*Azadiractaindica*	Leafs	Skinulcers,diabetes
2	Gritkumari	*Aloeverra*	Leaves	Constipation,diabetes
3	Telakochu	*Cocciniagrandis*	Leaves	Kidney stones,fatigue
4	Pathorkuchi	*Kalanchoepinnata*	Leaves	Intestinal problems,dysentery
5	Thankuni	*Centellaasiatica*	Leaves	Anxiety,hypertension,arthritis
6	Ulotkombol	*AbromaAugusta*	Leaves	Gonorrhea, diabetes,headacheswithsinusitis.

7	Arjun	*Terminaliaarjuna*	Bark	Chronicfever,Sinusitis,cough,urine retention
8	Durbaghas	*Cynodondactylon*	Whole plant	Pricklyheat,digestivedisorder,diabetes.

We collected most plant material (leaves, bark) from different sides of Jessore University ofScience 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 plantmaterialwithtap water,grindedwithsterile mortar andaddingdistilledwater gradually.Bysqueezingplantextract,wereserveitinglassbottle.
Thenfilteredwithsyringefilterandstoredin glass tube.

2.4 Biofilmassays

Biofilm formation was studied in glass test tubes. In each test tube 5ml of nutrient broth mediumwas inoculated with 20 µl of an overnight culture of the chosen bacterium and we added 100µl ofextract.Asacontrol,thesamevolumesofovernightculturewereused.Testtubeswereincubated at37°C for24hoursand then thebiofilmsstained 2%crystal violetdye.

2.5 Stainingtechniques

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. Toobtain quantitative data, the dye was solubilized with 70% ethanol and color intensity wasmeasuredin a spectrophotometer asabsorbanceatawavelengthof 540nm.Biofilmreduction concentration weremeasuredbyusingfollowingequation:

- Biofilm inhibition/eradication (%) = [1 − (OD540nm of test compound) / (OD540nm of control)] × 100%.

2.6 Agarwelldiffusionmethod

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 (100µl)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 8mm. Agar plates are then incubated at 37^0C, and the outcome is then observed.

2.7 Minimuminhibitoryconcentration

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 100µl plant extracts were added into 5ml nutrient broth containing test-tubes.Then the tubeswere incubated at 37°C for 24 hours. After incubation, planktonic cells concentrations weremeasuredat 540nm byspectrophotometerusingfollowingequation:

- Biofilm inhibition/eradication (%) = [1 − (OD540nm of test compound) / (OD540nm of control)] × 100%.

2.8 Swimmingmotility

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 Biofilmassays

Firstwetestedthe effectofadding*Cocciniagrandis,Terminaliaarjuna*and*Centellaasistica,C.dactylon,Aloeverra,Kalanchoe pinnata,Abromaaugustum,Azdiractaindica*plantextractson *S.entericaandS.aureus*biofilmformationinglasstubes(100 µlof extract in 5 ml). Bacterial cultures were observed after 24-hour incubation and then stainedwith2% crystal violet as described in Materials and Methods. *S.enterica and S.aureus*normallyforms a ring on the control tube surface. Figure: 1(a)and 1(b)displays the outcomes from some extracts.

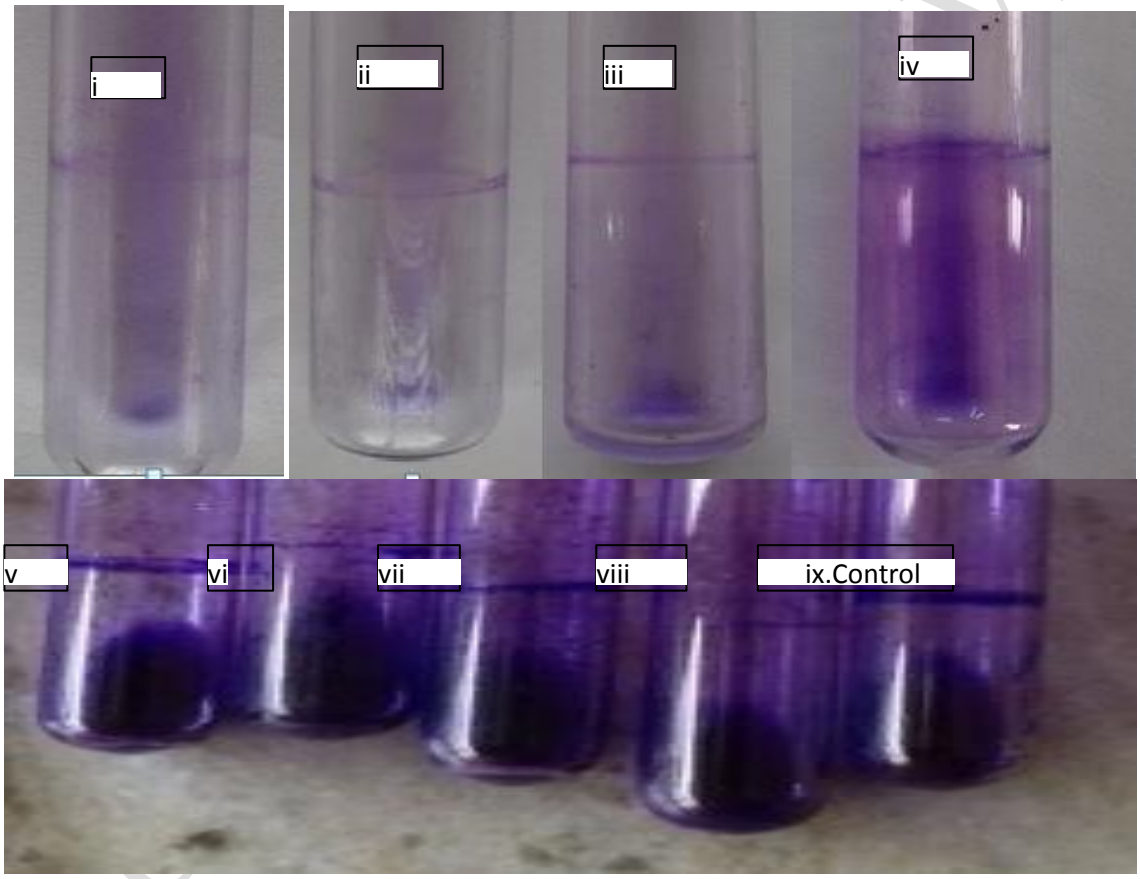


Figure 1(a): Formation *of S.enterica*biofilm in the presence *of plant extracts: (i) T.arjuna,(ii) C.grandis(iii)C.asiatica(iv)C.dactylon(v)Aloeverra(vi)Kalanchoepinnata(vii)Abromaaugustum(viii)Azdiracta indica(ix)Control(noextracts)*

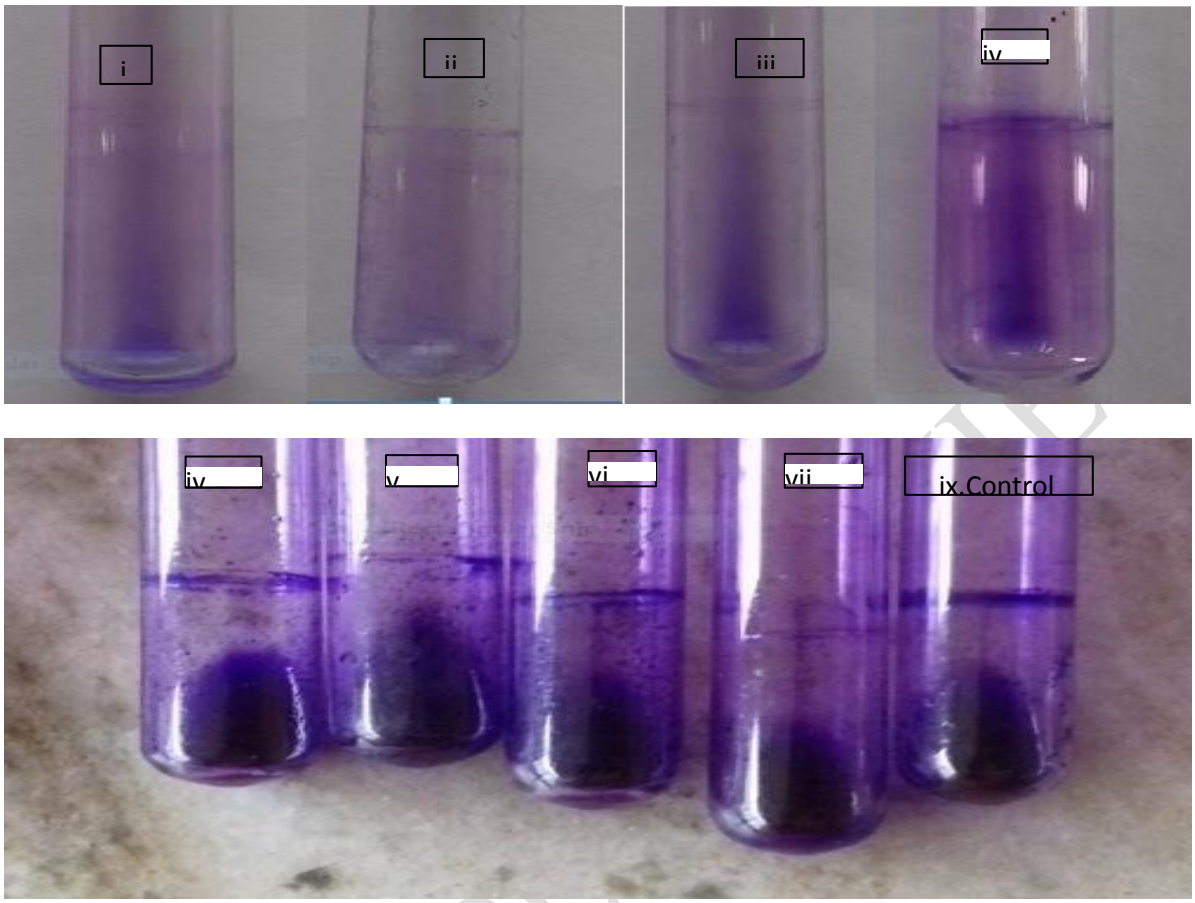


Figure1(b):*S.aureus*biofilm formation in the presence of plant extracts:(i*) T.arjuna*(ii) *C.grandis*(iii)*C.asiatica*(iv)*C.dactylon (v)Aloe verra (vi)Kalanchoepinnata (vii)Abromaaugustum(viii)Azdiractaindica(ix)*Control(noextracts)

After staining,more visible color was observed in *Salmonella enterica* and *Staphylococcusaureus*control tubes. In thecaseof *S.enterica*,colorintensityof theArjun containtube waslessclear 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, Kalanchoepinnata,Abromaaugustum, Azadiractaindica*we observed spread of biofilm. There is less stainingwithextract*T. arjuna* than in the control.Also in the case of *S. aureus*colorintensity ofthe*Cocciniagrandis* containing tube was less clear than the other extracts containing tube. Afterstaining, with extract *T. arjuna*and*C. asiatica* some of the biofilm hasshed. With extracts*C.dactylon, Aloe verra, Kalanchoepinnata, Abromaaugustum, Azadiractaindica*we observedspreadof biofilm. Thereis less stainingwithextract*C. grandis*thanin the control.

3.2 Antibiofilmpotentialofplantextracts

Inthisstudy,anti-biofilm(biofilmreduction)potentialof*T.arjuna,C.grandisandC.asiatica*plantextracts was also evaluatedand other extracts are not evaluated because they haveno effect for biofilm reduction assay. Extracts of *Terminaliaarjuna*displayed most potent S.enterica biofilm reduction (91.75%) and extracts of *Cocciniagrandis*and *Centellaasiatica*alsoreduced89.54%and 80.21%biofilm respectively.

Table2: Reductionof*Salmonellaenterica*biofilmonDefinePlantextracts.

Nameof theorganism	**Name of theextracts**	**Amountof culture**	**Amountofextracts**	**Optical density**	**%BIC**
Salmonellaenteric	Control (no extract)	20µl	0	2.40	0.00
Salmonellaenteric	*Cocciniagrandis*	20µl	100 µl	0.251	89.542
Salmonellaenterica	*Terminaliaarjuna*	20µl	100 µl	0.198	91.75
Salmonella enterica	*Centellaasiatica*	20µl	100 µl	0.475	80.208

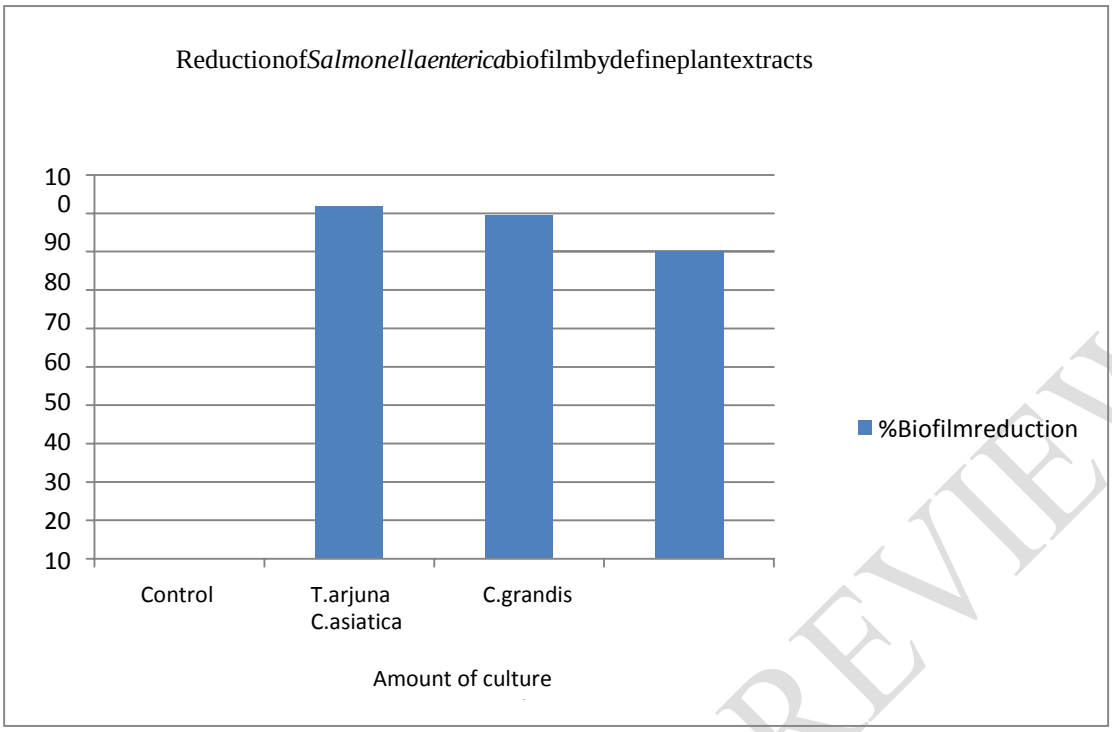


Figure 2(a): Graphicalrepresentationof reduction*S.enterica*biofilmondefineplantextracts

Also extracts of *Cocciniagrandis*displayedmostpotent*S. enterica* biofilm reduction (86.88%)andextractsof*Terminaliaarjuna*and*Centellaasiatica*alsoreduced(80.97% and82.27%)biofilmrespectively and other extracts are not used because they have no effect for this biofilm reductionassay.

Table3: Reductionof *Staphylococcusaureus* biofilmonDefinePlantextracts.

Name of theorganism	Name of theextracts	Amountof culture	Amountof extracts	Optical density	%BIC
Staphylococcusa ureus	Control(noextract)	20µl	0	1.76	0.00
Staphylococcus aureus	*Cocciniagrandis*	20µl	100 µl	0.231	86.875
Staphylococcusa ureus	*Terminaliaarjuna*	20µl	100 µl	0.335	80.966
Staphylococcus aureus	*Centellaasiatica*	20µl	100 µl	0.312	82.273

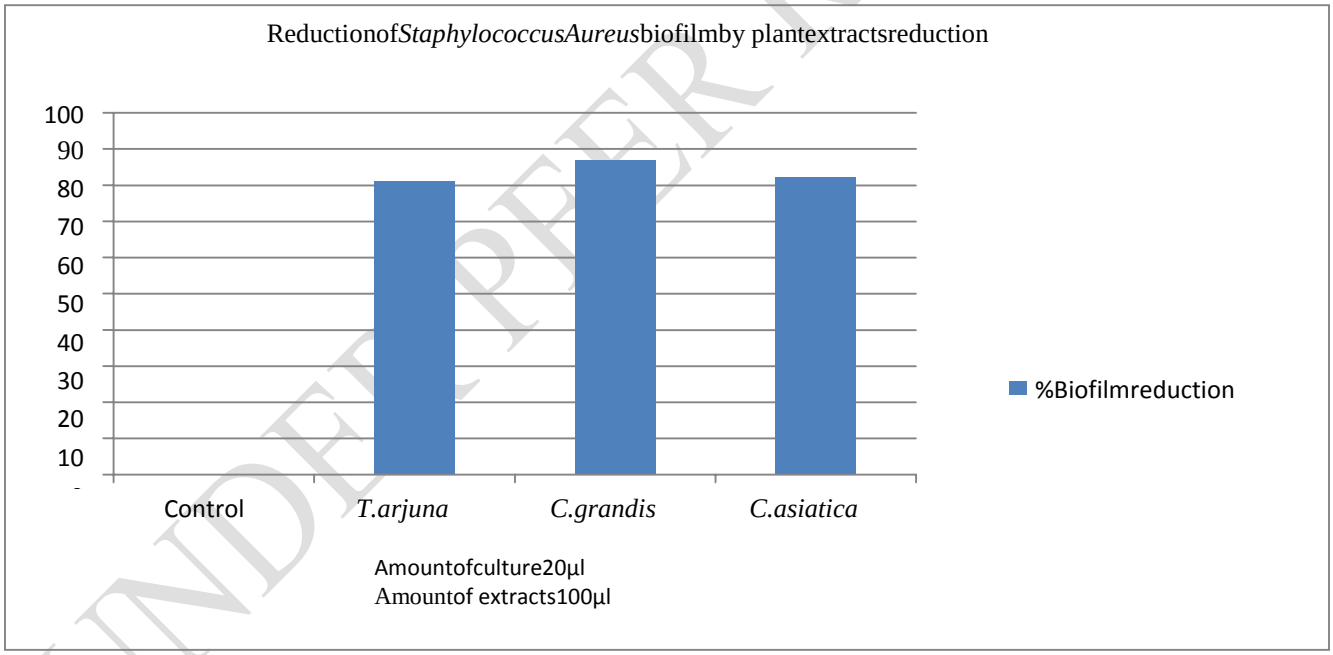


Figure 2(b): GraphicalrepresentationofreductionS.*aureus*biofilmondefineplantextracts

3.3 MinimumInhibitionConcentration(MIC)

It wasfoundthattheMICof*Centellaasiatca,Terminaliaarjuna,Cocciniagrandis*were2.27%,1.60%,1.13%for theSalmonella enterica.

Table4(a): Minimum Inhibition Concentration of *Salmonella enterica* on definePlant extracts.

Name of theorganism	Name of theextracts	Amount ofculture	Amount ofextracts	Optical density	%MIC
Salmonella enteric	Control(no extract)	20µl	0	4.40	0
Salmonella enteric	*Cocciniagrandis*	20µl	100 µl	4.35	1.13
Salmonella enteric	*Terminaliaarjuna*	20µl	100 µl	4.33	1.60
Salmonella enteric	*Centellaasiatica*	20µl	100 µl	4.30	2.27

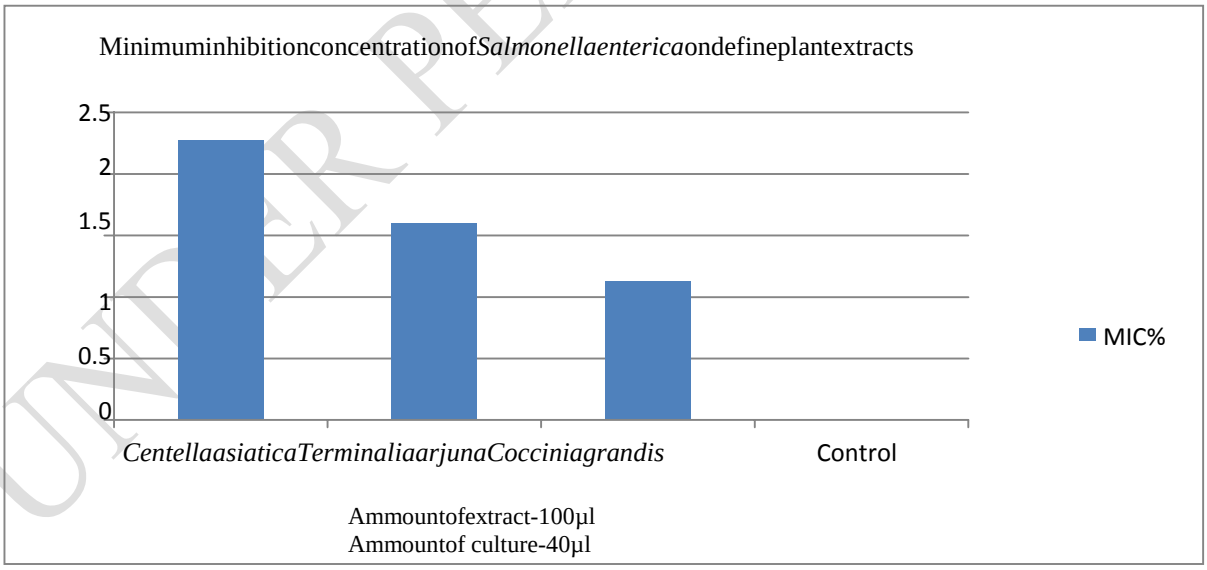


Figure 3(a): GraphicalrepresentationofMIC*S.enterica*ondefineplantextracts.

ItwasfoundthattheMICof*Centellaasiatca,Terminaliaarjuna,Cocciniagrandis*were2.48%, 1.35%, 2.93%for the*Staphylococcusaureus*.

Table 4(b): Minimum Inhibition Concentration of *Staphylococcus aureus* onDefinePlant extracts.

Name of theorganism	Name of theextracts	Amount ofculture	Amount ofextracts	Opticald ensity	%MIC
Staphylococcus aureus	Control(no extract)	20µl	0	4.43	0
Staphylococcus aureus	*Cocciniagrandis*	20µl	100 µl	4.30	2.93
Staphylococcus aureus	*Terminaliaarjuna*	20µl	100 µl	4.37	1.35
Staphylococcus aureus	*Centellaasiatica*	20µl	100 µl	4.32	2.48

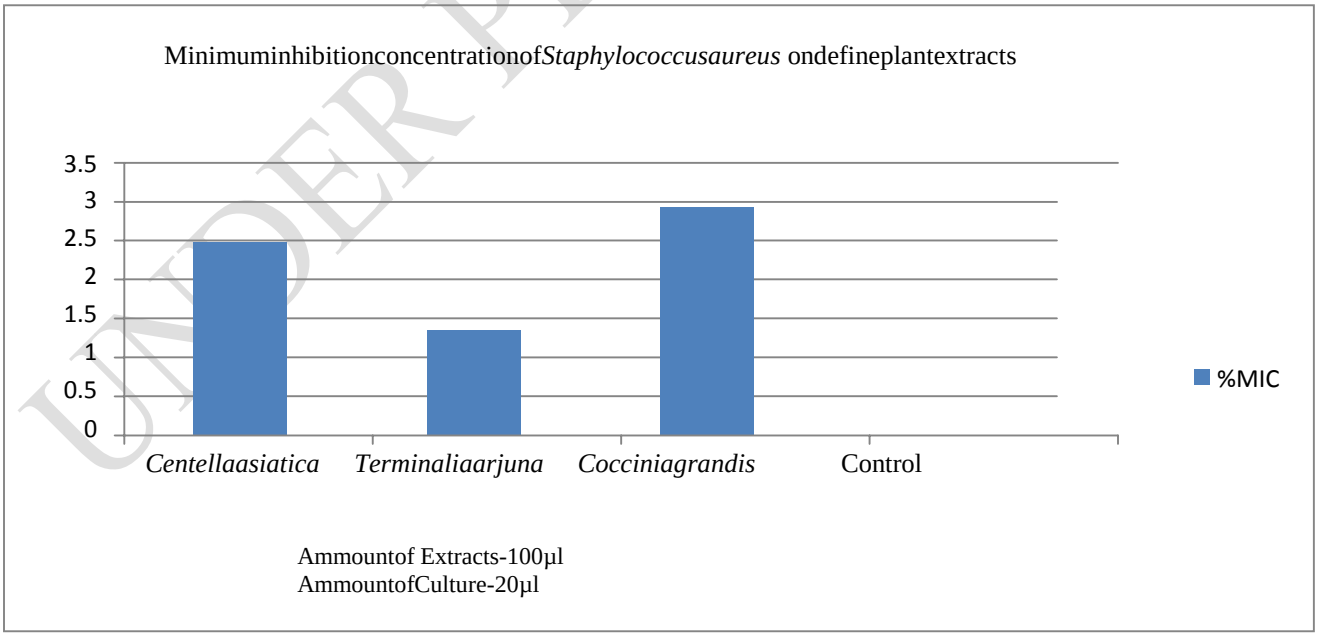
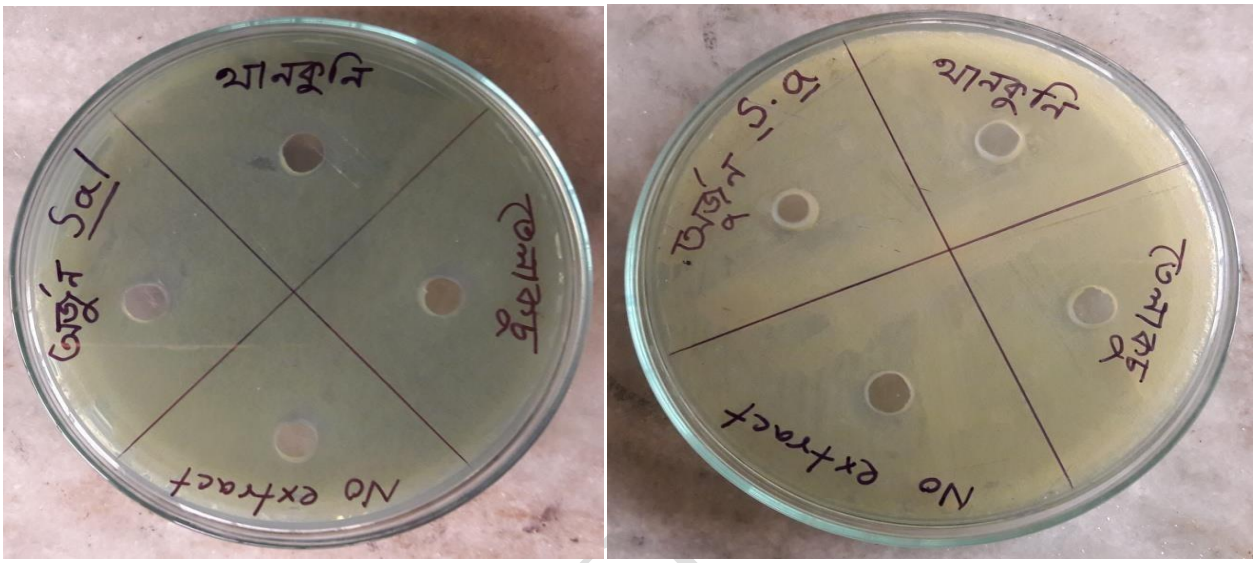


Figure 3(b): GraphicalrepresentationofMICof*S.aureus*ondefineplantextracts.

3.4 Antiplantoniceffect

Ourdesiredplantextractshavenoantiplantonic effectagainstthetestorganism.Forthisreason,thereis no zoneof inhibition werefoundin thepetri plate byusingagarwell diffusionmethod.Showin figure:4



(i) **(ii)**

Figure 4: Antiplantoniceffectofdefineplantextractsagainst(i)*S.enterica(*ii)*S.aureus*

3.5 SwimmingMotality

We tested if our desire plant extracts altered motility of S.aureus& 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 Figure:5(a)

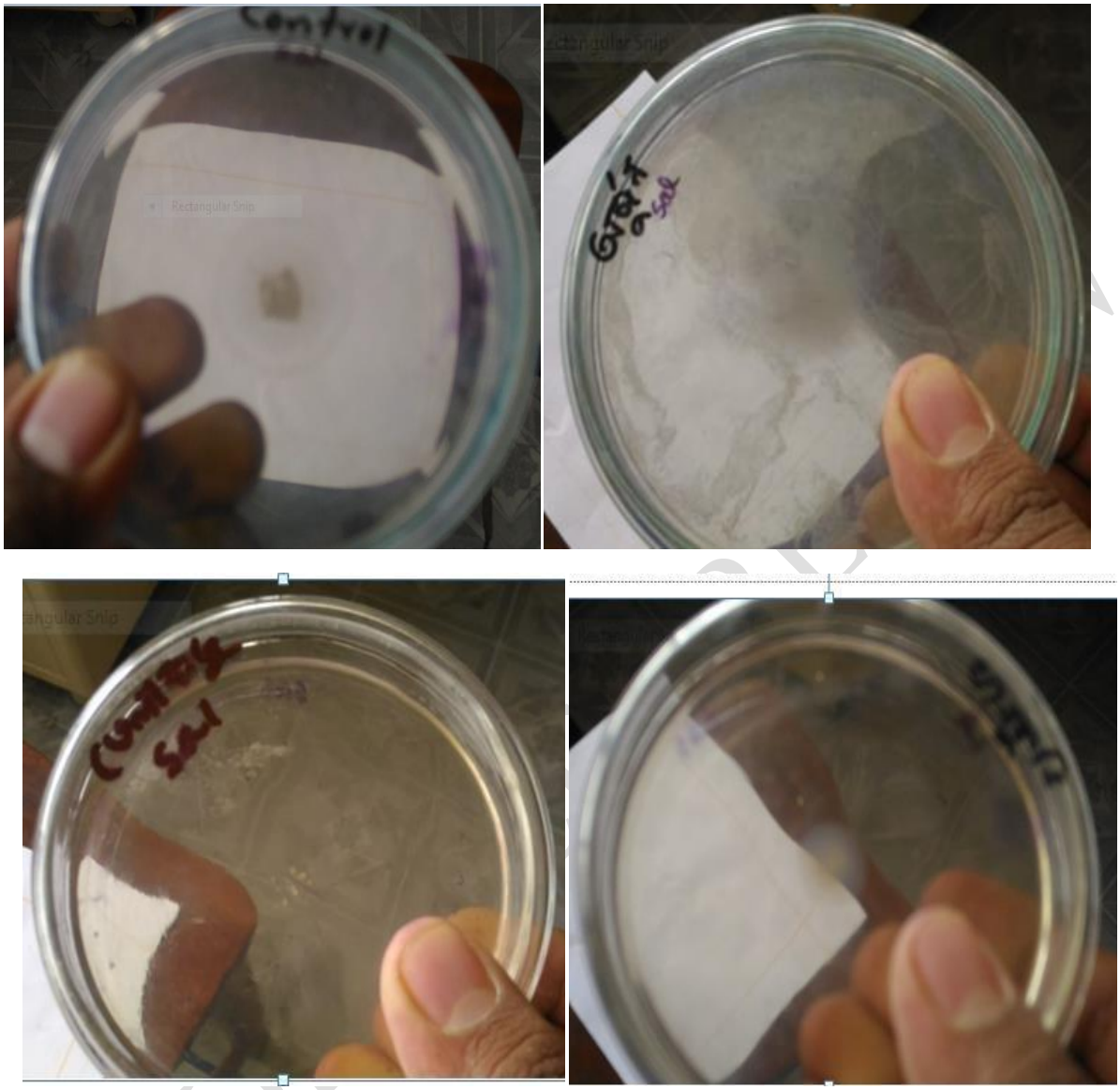


Figure 5(a): Swimmingmotilityof S. entericain thepresenceof plantextracts.(i)control(noextracts)(ii)*T. arjuna*(iii)*C. grandis*(iv)*C.asiatica*

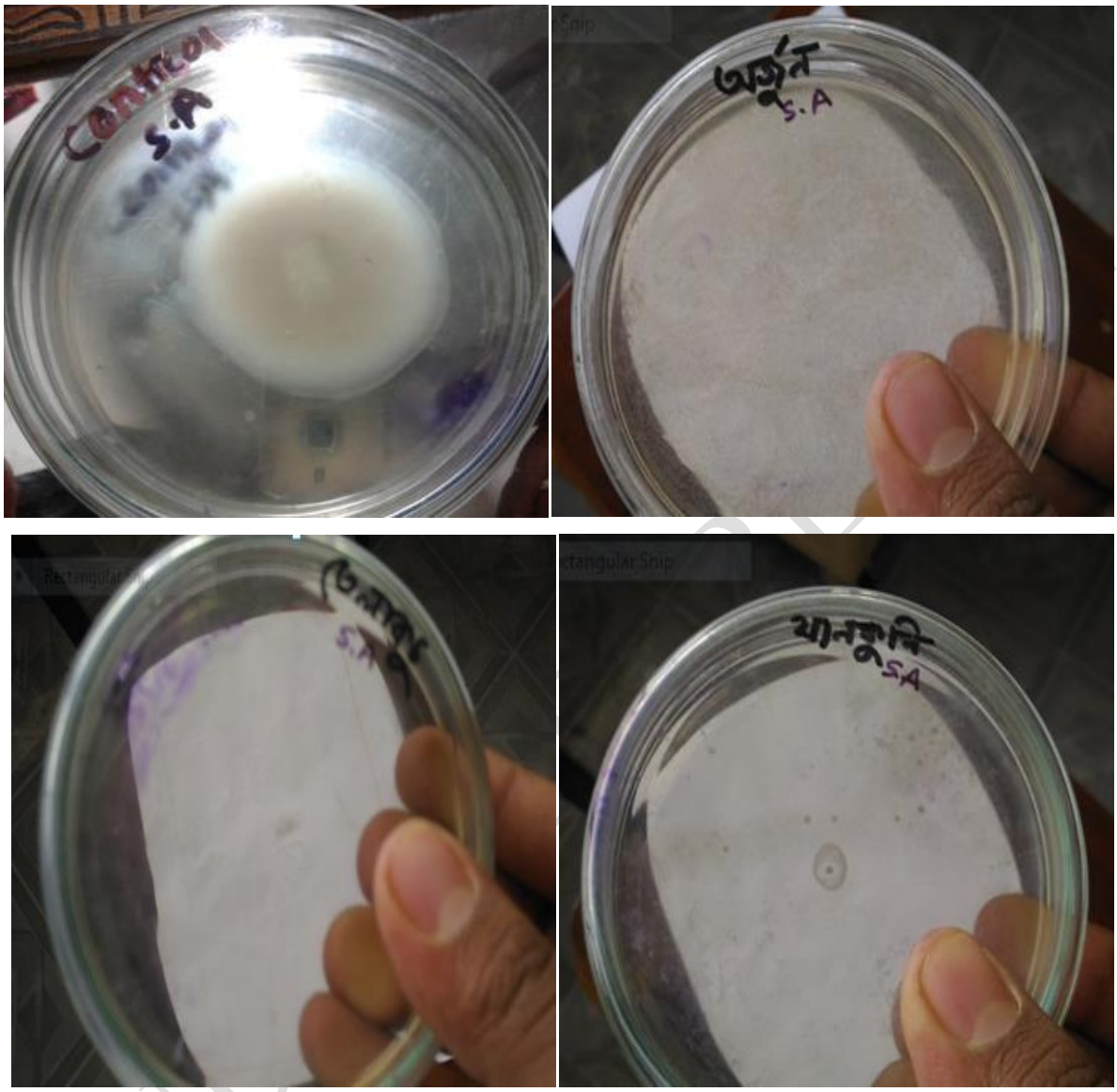


Figure 5(b):Swimming motility of *S.aureus* in the presence of plant extracts. (i)Control plate(no extract) (ii)*T.arjuna* (iii) *C.grandis* (iv) *C.asiatica*.

Table5(a): Diameter ofswimming motility halo of *Salmonella enterica* in the presence of differentextracts

Name of theOrganism	Nameof theExtract	Amount ofExtract	Amount ofCulture	Incubation Time(Hour)	Diameter ofHalo(cm)
Salmonella enterica	Control(noextract)	0.00	One loop	16 h	3.2cm
Salmonella enterica	*Cocciniagrandis*	300µl	One loop	16 h	0.00cm
Salmonella enterica	*Terminaliaarjuna*	300µl	One loop	16 h	0.00cm
Salmonellae nterica	*Centellaasiatica*	300µl	One loop	16 h	0.50cm

Table5(b): Diameterofswimmingmotilityhaloof *Staphylococcusaureus* inthepresenceofdifferentextracts

Nameof the Organism	Nameof the Extract	Amount of Extract	Amount of Culture	Incubation Time(Hour)	Diameterof Halo(cm)
Staphylococcus aureus	Control(no extract)	0.00	One loop	16 h	3.5cm
Staphylococcus aureus	*Cocciniagrandis*	300µl	One loop	16 h	0.00cm
Staphylococcus aureus	*Terminaliaarjuna*	300µl	One loop	16 h	0.00cm
Staphylococcus aureus	*Centellaasiatica*	300µl	One loop	16 h	0.30cm

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 sideeffectslikecommercialdrugs.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.

However, in our study the biochemicalcompositionofthe *Salmonella enterica*&*Staphylococcus aureus* biofilmmatrixhasbeenhighly reducedby the watery plant extract *Cocciniagrandis, Terminalia arjuna&Centellaasiatica.* Out ofthese 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 itabove 80%*. *T.arjuna*bark is very effective for gastrointestinal diseases and *S.enterica*is mostlyresponsible for these disorder. The aqueous bark extracts of *T. arjuna* lacked antifungal activityagainst *C. albicans* and antibacterial activity against*S. aureus* that was found sensitive both tothe hot and cold aqueous bark extracts[23]. So the extracts strongly fightwith this organism and reduces its biofilm mostly. In the case of *S.aureus, C.grandis*is

veryeffectiveandreduces86.875%biofilm,otherplantsalsoreduceabove80%*S.aureus*biofilm. *S.aureus*isanopportunisticpathogenbutinimmunesuppresspatienttheyproducemainly skin disorder and *C.grandis*leaf paste is very useful for that disorder,So it is said that allplant extracts are very strongly reduces the biofilm of these pathogenic organisms. Peppermintessential 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 activityagainst test organisms. Our desired plant extracts have no anti-planktonic activity against testorganisms.For that reason,planktonic cell concentration in the extract containing tubes are verysimilar with control tubes(no extract). *Centellaasiatica* leaves (water soluble) showed best anti-microbial activities against Mycobacterium spp.[27]. But they reduce the swimmingmotility of these organisms.

Swimming motility is the key factor for the biofilm formation withoutit, organisms are unable to produce biofilm in the surfaces[28]. All organismsproduce biofilm except genetically modified organisms but without motility they are stronglyunable to form it[29]. Delft University of TechnologyBoth free and chitosan coated plant extracts inhibited biofilm formation by *E. coli,* and enhancedeffect on biofilm inhibition was recorded in polymer coated extracts of the all the tested plants[30,31]. This result indicated that *T.arjuna, C.grandisand 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 causesillnesses in humans and many animals, such as typhoidfever,paratyphoidfever,andthefoodborneillnesssalmonellosis[32].Itcanbeconcludedthat*Cocciniagrandis,Terminaliaarjuna,Centellaasiatica,Cynodondctylon,Alovera,Abromaaugustum,Azardirchataindica,Kalanchoepinnata*isanimportantsourceofmanypharmacological and medicinally important chemicals. The present study was carried out ona preliminary basis, in order to identify the plants that capable of reduce *S.enterica*and *S.aureus*biofilms.Theextractsof*Cocciniagrandis,Centellaasiatica,Terminalia arjuna* wereabletoinhibit biofilm of *Staphylococcus aureus& Salmonella enterica* biofilm. By understanding thetrue mechanism ofanti-biofilmeffectof these extractscanhelpto fight againstinfectionsdueto*Staphylococcusaureus&Salmonellaenterica*.Theuseoftheseplantextracts astherapeuticagentcouldhelptosavecostandreducechemicaldrugtoxicitiesorsideeffects.Further experiments are required to study more and more extracts in detail, their potentialasanti-biofilmeffecton healthand componentanalysis of theseplantextracts.

6. REFERENCES

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