European Journal of Molecular & Clinical Medicine
ISSN 2551-8260 Volume 07 , Issue 08 , 2020

Efficacy of selected three plants extracts in controlling the survival of Aeromonas hydrophila, Vibrio parahaemolyticus, Salmonella typhi, Escherichia coli and Bacillus subtilis on wood, plastic iron and stainless steel surfaces

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Introduction

Food soil is the unwanted matter on food contact surfaces which may be visible or invisible. In slaughterhouses fats and proteins are the most common types of soil which is a source of bacterial contamination and further results in cross contamination of meat. Personnel involved in cleaning purpose should have a background knowledge regarding the nature of soil before selecting a detergent. Improper usage of these sanitizers and disinfectants leads to develop resistance among food borne pathogens, which may be innate, apparent or acquired. Due to the non-specificity of sanitizers, the development of resistance is mostly caused by innate factors (Russell, 1997) which are chromosomally controlled associated with organisms. The mechanisms include impermeable cellular barriers preventing penetration of the sanitizer, cellular efflux (mechanisms inside the cell pump compounds out), and lack of a biochemical target for antimicrobial attachment or microbial inactivation and inactivation of antimicrobials by microbial enzymes (Davidson *et al.,* 2002 and Bower *et al.,* 1999). In this regard, due to unsuccessful disinfection processes and emerging resistance, conventional control measures becoming ineffective, necessitating the development of new strategies (Valeriano *et al.,* 2012). Growing negative consumer perception towards synthetic disinfectants led to the search of natural al[ı]ternatives.

Review of Literature

Food industry approaches on different strategies to inhibit the growth and survival of these biofilms which concentrates on both physical and chemical methods, as the microbes attached were resistant to sanitation (Gilbert *et al.,* 2002). Quaternary ammonium compounds (QACs) like benzalkonium chloride (BC) are commonly used in food-processing units as disinfectants (Moen *et al.,* 2012) and will target the inner cytoplasmic membrane and the outer membrane of gram negative bacteria. But the prolonged use of these disinfectants leads to the development of resistance (extrinsic /intrinsic) among bacteria (Hegstad *et al.,* 2010) which is of severe concern as in the case of antibiotic resistance (Langsrud *et al.,* 2003 and Sidhu *et al.,* 2001). The impermeability of the outer membrane of gram negative bacteria provides more resistance towards the action of disinfectants than gram positive bacteria (McDonnell and Russell, 1999). The resistance acquired by *E. coli* and *Pseudomonas aeruginosa* has been associated with the alterations made in the membrane composition and the rapid resistance acquisition to QACs in *E. coli* may be due to the changes in lipopolysacchride composition and reduction in OmpF (Ishikawa *et al.,* 2002). An

active efflux of cationic surfactants has been reported in *Staphylococci* (Heir *et al.,* 1998) and *Listeria monocytogenes* (Aase *et al.,* 2000). Sidhu *et al.,* (2001; 2002) observed a genetic linkage encoding resiatance towards QACs and b-lactams in *Staphylococci*. Braoudaki *et al.,* (2004, 2005); Langsrud *et al.,* (2003) and Sidhu *et al.,* (2001) reported that bacterium develops resistance to high levels of benzalkonium chloride and cross resistance to other antimicrobials.

These resistance of bacteria towards antibiotics and disinfectants results in an increased concern regarding the effectiveness and safety, which leads to the search of novel means for the successful removal of biofilms which were safe to consumers as well as to the environment. Generally different types of plants were used in our daily diet. In addition to the flavour and fragrance qualities, many of the spices and herbs were evaluated for their antibacterial potency (Ceylan *et al.*, 2004).

The hexane, ethyl acetate, ethanol and hydro-alcoholic extract of *Garcinia gummi-gutta* fruit rind were evaluated by Shivakumar *et al.* (2013) for antibacterial activity and revealed that the ethyl acetate extract of *G. gummi-gutta* exhibits better antibacterial activity where as hexane extract showed on activity against any of the test pathogens. A review by Tharachand *et al.* (2013) documented the root contains xanthone called garbogiol, bark with benzophenones such as garcinol and isogarcinol and possess antioxidant, antihelmintic, anticattarhal, anti-cancer and antimicrobial activities. Preliminary phytochemical analysis of the secondary metabolites in the leaf of *Garcinia gummi-gutta* were studied by Madappa *et al.* (2012) and noted the presence of high content of alkaloids, tannins, phenolic flavonoids, flavonoids, carbohydrates and proteins.

The leaf extracts of *T. indica* were subjected to antimicrobial analysis by Escalona *et al.* (2010) and Gumgumjee *et al.* (2012) observed that the leaf can check the microbial growth, also reported the presence of flavanoidal glycosides- Orientin and Vitexin. The potential of fruit extract of *T. indica* in controlling the growth of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were confirmed by the study conducted by Chowdhury *et al.* (2013). Crude fruit extracts of *Tamarindus indica* showed potency against *Pseudomonas aeruginosa* at varying concentrations as reported by Donkor *et al.* (2014).

The study of Oluduro (2012) on the antibacterial activity of *Moringa oleifera* in controlling bacteria and fungi reveals the potential for using the leaves as an alternative therapy for wounds and certain fungal infections. Preliminary phytochemical screening of *M. oleifera* by Sharma *et al.* (2013) and Onyekaba *et al.* (2013) reveals the presence of phenolics, triterpenoids, cardiac glycosides, steroid, alkaloids, phlobatannins, cardiac glycosides, reducing sugars and saponin in the plant extract and also reported as a good source of antimicrobials and natural antioxidants (Govardhan *et al.,* 2013). Rockwood *et al.* (2013) investigate pragmatic extraction techniques for seed and leaf extracts of *M. oleifera* to mention the difference in the inhibition of microbial growth and reported that there is no difference between crude and sophisticated extracts. The *in vitro* antimicrobial activity by disc diffusion method and MIC method against *Staphylococcus aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris* were investigated by Sumathy *et al.* (2013) and observed that the flavanoid rich fraction of *Moringa oleifera* was effective against *S. aureus* and *B. subtilis*. Thin layer chromatography bioassay was used by Oluduro *et al.* (2010) against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Cladosporium cladosporioides*, and *Penicillium sclerotigenum* to assess the antimicrobial activity. Characterization and identification of the extract revealed the

occurrence of three bioactive compounds: 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, methyl *N*-4-(α-L-rhamnopyranosyloxy) benzyl carbamate, and 4-(α -D-glucopyranosyl-1→4- α -L-rhamnopyranosyloxy)- benzyl thiocarboxamide.

Use of various plant extracts in controlling the survival of these organisms on the processing surfaces is seriously being worked. In the current scenario, the emerging resistance among bacteria and the adverse effects of chemical disinfectants is a serious menace in meat processing units. In this context of emerging resistance, we have set the following objectives.

1. To evaluate the antibacterial activity of methanolic extract of the selected plants.
2. To assess the antibacterial activity of commonly used disinfectants and antiseptics using agar well method.
3. To determine the minimum inhibitory concentration of the selected plant extracts.
4. To determine the efficacy of selected plant extracts in controlling the growth of food borne pathogens on different meat cutting surfaces.

Materials and methods

Antibacterial activity of methanolic extracts of selected plants using agar well method

Preparation of Extracts

The powdered leaves and fruit of *T. indica, G. gummi-gutta* and *M. oleifera* (20 g) were extracted in 200 ml of absolute methanol in a Soxhlet apparatus for 8 hrs. The extract was filtered and was concentrated using rotary vacuum evaporator. The dried powders of the extracts were refrigerated and were reconstituted with methanol for further analysis.

Antibacterial assay

Test pathogens were inoculated in nutrient broth and kept for incubation at 37ºC for 6-8 hours. The bacterial cultures were swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Using a sterile cotton swab, the bacterial cultures enriched in sterile nutrient broth Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter (Srinivasan *et al.,* 2001). Using a micropipette, 100 micro litres of plant extracts were added to different wells in the plate. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition zones was measured in millimetres. Inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity. Diameters between 12 and 16 mm were considered as moderately active, and greater than 16 mm were considered as highly active (Bauer *et al.,*1966).

Antibacterial potential of some commonly used disinfectants, detergents and antiseptic ointment

The bacterial cultures enriched in sterile nutrient broth for 6-8 hours at 37ºC were swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Agar wells were prepared with the help of sterilized cork borer with 10mm diameter (Srinivasan *et al.,* 2001). Selected disinfectants such as ethanol and methanol, detergent, dish wash bar and colloidal silver gel used in wound dressing were used. Using a micropipette, hundred micro litres of these disinfectants were added to different wells in the plate. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition zones was measured in millimetres. Inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity. Diameters between 12 and 16 mm were considered as moderately active, and

greater than 16 mm were considered as highly active (Bauer *et al.*,1966).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined using colorimetric broth microdilution method (Abate *et al.*, 1988 & Trila *et al.*, 2014). Bacterial strains *E. coli, S. typhi, V. parahaemolyticus, A. hydrophila* and *B. subtilis* were cultured in nutrient broth at 37^oC for 24 hours. The inoculum density was adjusted to 1×10^6 cfu/ml. The extracts were prepared in different concentration (between 1 mg/ml to 50 mg/ml), by dissolving the powdered extracts in sterile distilled water. The 96 well microplates were prepared by dispensing 0.09ml of Muller Hinton broth and 0.01ml of inoculums into each well. The plant extracts with varying concentrations were added to the wells and were incubated at 37^0C for 24 hours. After incubation, 0.02ml of viability indicator (3-{4.5-dimethylthiazol-2-yl}-2, 5-diphenyl tetrazolium bromide (MTT) were added in the well and incubated for one hour to allow the viable bacterial strains to metabolize the yellow dye into formazan, with a purple colour. The MIC value was considered as the concentration of the well with no change in colour from yellow to purple.

Efficacy of plant extracts in controlling bacterial growth on various cutting surfaces

Preparation of crude extract

Based on the results from the preliminary study, the leaf and fruit extract of *Tamarindus indica* and *Garcinia gummi gutta* and leaf extract of *Moringa oleifera* were finalised for the efficacy studies (Fig. 5.1). The officinal parts (100 g) were crushed in a sterile blender with 100ml of sterile distilled water and the resultant slurry was filtered through cheesecloth.

Efficacy of plant extract

For assessing the efficacy of these plant extracts, 0.5 ml of the inoculum containing 10^6 cfu ml^{-1} of the bacterial suspension was spread on to the entire surface of autoclaved wooden, plastic and metal blocks. The excess water on the surface of the blocks was removed by using desiccator. The plant extracts prepared were flooded over the seeded blocks and kept at room temperature. The blocks were removed at regular intervals (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min) and were then dipped directly into test tubes containing sterile distilled water. One hundred micro liters of bacterial suspension from the distilled water were spread after incubation on to the selective agar plates and were incubated at room temperature for 24 hours and the number of bacterial colonies was counted. The respective controls of sterile and contaminated wooden, plastic and metal blocks were maintained (Suresh *et al.,* 2004). The percentage survival rate was calculated by the following equation

$$\text{Percentage survival (\%)} = \frac{\text{Number of colonies survived at time 't'}}{\text{Number of colonies at time '0'}} \times 100$$

T. indica


G. gummi-gutta *M. oleifera*

Figure 5.1. Plants selected for efficacy studies

Result and Discussion

Antibacterial potential of methanolic extracts of *G. gummi-gutta, T. indica* and *M. oleifera* using agar well method

Officinal parts of the plants which selected for the efficacy study were extracted with methanol and were further evaluated for their antibacterial potential against common food borne pathogens such as *S. typhi, A. hydrophila, E. coli, V. paraheamolyticus* and *B. subtilis.* The results were represented in the Table 5.1. The diameter of zone of inhibition was very high for the fruit extract of *G. gummi-gutta* against *S. typhi* and *V. parahaemolyticus* (Figure 5.2). Shivakumar *et al.* (2013), Semwal *et al.* (2015), figured out the ethyl acetate, ethanol and hydro-alcoholic extracts from the fruit rind which showed bactericidal activity against *E. coli, B. subtilis* and *S. aureus* with inhibition zone diameters ranging from15 to 34 mm. However, our observations revealed much higher activity.

The methanolic fruit extract of *T. indica* also exhibited very good bactericidal activity in against of *S. typhi, A. hydrophila* and *E. coli*. The study conducted by Prabhu *et al.* (2011), Nehad *et al.* (2012), Elumalai *et al.* (2015) and Rothu *et al.* (2015) revealed the potential of *T. indica* fruit in controlling *E. coli* and *B. subtilis* which agrees with findings of our present study. The antimicrobial activity of *T. indica* may be attributed to the presence of compounds such as phenolic acids and flavonoids (Trila *et al.,* 2014).

The aqueous extract of *M. oleifera* leaf do not posses bactericidal property in controlling the growth of *A. hydrophila*, *E. coli* and *B. subtilis*, however the results of the methanolic extracts reveals its potential (moderate activity) to control these strains. Peixoto *et al.* (2011) analysed the bactericidal activity of aqueous and alcoholic leaf extract and reported that *E. coli* was resistant while *V. parahaemolyticus* as susceptible to the activity of these extracts. This study supports our findings in controlling the growth of *V. parahaemolyticus*.

Though the aqueous extract of *G. gummi-gutta* and *T. indica* leaves do not owe antibacterial property against *A. hydrophila* and *B. subtilis*, but methanolic extracts check the growth of these pathogens.

Table. Antibacterial activity of methanolic extracts of selected plants using agar well method

Plant	**Officinal Part**	**Diameter of zone of inhibition in mm**				
		S. typhi	***A. hydro***	***E. coli***	***V. para***	***B. sub***

M. oleifera	Leaf	26	14	12	30	14
G. gummi-gutta	Leaf	27	12	27	29	24
G. gummi-gutta	Fruit	43	29	30	43	41
T. indica	Leaf	31	24	27	22	20
T. indica	Fruit	42	29	28	30	14

S. typhi – Salmonella typhi, A. hydro – A. hydrophila, E. coli- Escherechia coli, V. para – Vibrio parahaemolyticus, B. sub – Bacillus subtilis

Antibacterial activity of commonly used disinfectants and antiseptic

The antibacterial activity of disinfectants like ethanol and methanol, detergent, dish wash bar and colloidal silver gel used in wound dressing were evaluated. The results revealed that all the test organisms were resistant towards all these disinfectants. No zone of diameter was observed (Fig: 5. 3). The results were represented in Table 5. 2.

Table Antibacterial activity of commonly disinfectants and antiseptics using agar well method

Disinfectants and antiseptic	**Diameter of zone of inhibition in mm**				
	S. typhi	***A. hydro***	***E. coli***	***V. para***	***B. sub***
Methanol	0	0	0	0	0
Ethanol	0	0	0	0	0
Dishwashing bar	0	0	0	0	0
Detergent	0	0	0	0	0
Colloidal silver gel	0	0	0	0	0

S. typhi – Salmonella typhi, A. hydro – A. hydrophila, E. coli- Escherechia coli, V. para – Vibrio parahaemolyticus, B. sub – Bacillus subtilis

Minimum inhibitory concentration (MIC) of the selected plants

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that can inhibit the growth of organisms. Determination of the MIC is very significant as it helps in confirming resistance of micro-organism against an antimicrobial agent and it monitors the activity of new antimicrobial agents. The lowest concentration of plant extract that completely killed the bacteria was taken as the Minimum Bactericidal Concentration or MBC.

Table MIC of selected plant extracts against food borne pathogens

Plant	Part used	Concentration of plant extracts in mg/ml				
		S. typhi	***V. para***	***A. hydro***	***E. coli O86***	***B. sub***
M. oleifera	Leaf	15	18	19	15	20
G. gummi-gutta	Leaf	17	18	34	28	22
G. gummi-gutta	Fruit	9	11	11	17	12
T. indica	Leaf	19	20	16	22	33
T. indica	Fruit	11	14	13	19	17

S. typhi- Salmonella typhi, V. para- Vibrio paraheamolyticus, A. hydro- A. hydrophila, E. coli- Escherechia coli, B. sub- Bacillus subtilis

The minimum inhibitory concentrations of the five extracts were represented in Table 5.3. The MIC value of *M. oleifera* leaf extract ranges from 15 mg/ml to 20 mg/ml. In controlling the growth of *S. typhi* and *E. coli*, 15 mg/ml were required. Highest concentration was observed for *B. subtilis* (20 mg/ml) and for *V. parahaemolyticus* and *A. hydrophila* (18 and 19 mm respectively). The leaf and fruit extract of *G. gummi-gutta* were observed for their minimum concentration required to check the pathogenic strains. The results for the fruit rind were observed to be very promising (MIC ranging from 9 mg/ml to 17 mg/ml). A concentration of 9 mg/ml was observed for *S. typhi*, 11 mg/ml for *V. parahaemolyticus* and *A. hydrophila*, 17 mg/ml and 12 mg/ml for *E. coli* and *B. subtilis* respectively. The highest concentration of leaf extract for checking the growth of *A. hydrophila* was observed as 34 mg/ml and the lowest as 17 mg/ml towards *S. typhi*. MIC study of the fruit pulp of *T. indica* revealed that only 11 mg/ml of the extract was needed to suppress the growth of *S. typhi* and highest concentration of 19 mg/ml control growth of *E. coli.* The MIC values of leaf extract of *T. indica* against various pathogens ranged from 16 mg/ml and 33 mg/ml.

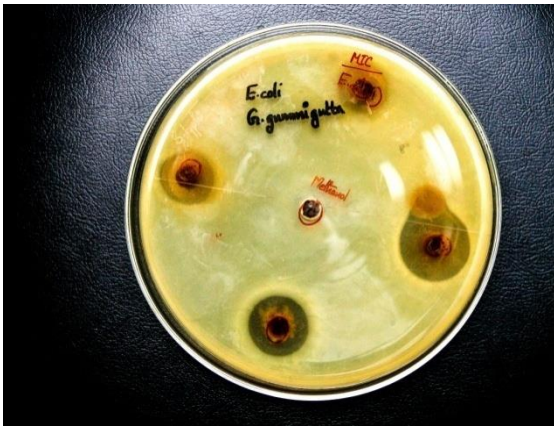

Figure Inhibition zones of methanolic extract of *G. gummi-gutta* against the test pathogenic strains

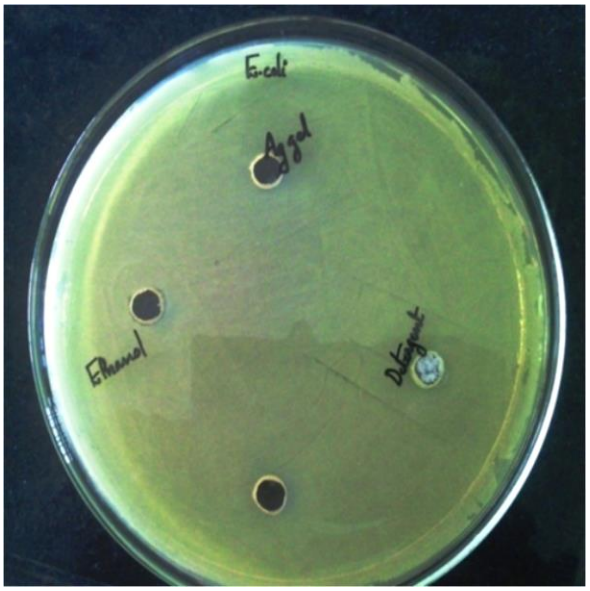

Figure Antibacterial activity of commonly used disinfectants and antiseptics

Efficacy of *M. oleifera*, *T. indica* and *G. gummi-gutta* as disinfectants

The plants selected for evaluating the efficacy in controlling food borne pathogens were leaf and fruit extract of *G. gummi-gutta*, *T. indica* and leaf of *M. oleifera*. The selections of these plants were based on the preliminary screening and the usage of these plants in our food preparations. This experimental study was set up with an intention to help the meat processing units to control the survival of food borne pathogens in an eco friendly manner. In this study the percentage inhibition of different pathogens with the application of plant extracts at different time intervals (1-10 minutes) were observed. The results of the efficacy of plant extracts was analysed using DMRT (Duncan's Multiple Range Test). The statistical significance was measured using one way ANOVA. The 'P value' was found to be <0.05, which is considered to be significant.

The antibacterial efficacy of selected plant extracts was evaluated by quantifying viable bacterial cells after the application of these extracts. The effect of each extracts was expressed as percentage survival between initial adhered cells and remaining viable cells after the application of extracts. The efficacy of the plant extracts were found to control the growth of selected pathogen on all the test surfaces.

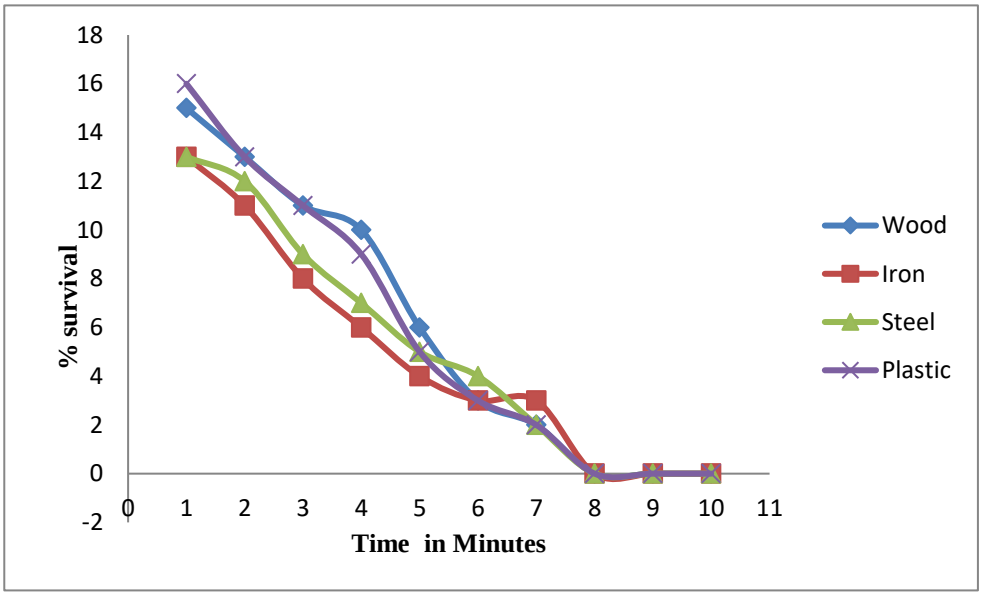

Figure Effect of leaf extract of *G. gummi-gutta* in controlling the growth of *V. paraheamolyticus* on different cutting surfaces

The efficacy of leaf extract of *G. gummi-gutta* in controlling the growth of *V. paraheamolyticus* on different cutting surfaces was depicted in the Figure 5.4. Up to seventh minute the colonies were observed on all the test surfaces and beyond which there was no growth.

S. typhi was found to be sensitive to the fruit extract of *G. gummi-gutta* and the growth was suppressed at the sixth minute itself on all surfaces (Figure 5.5).

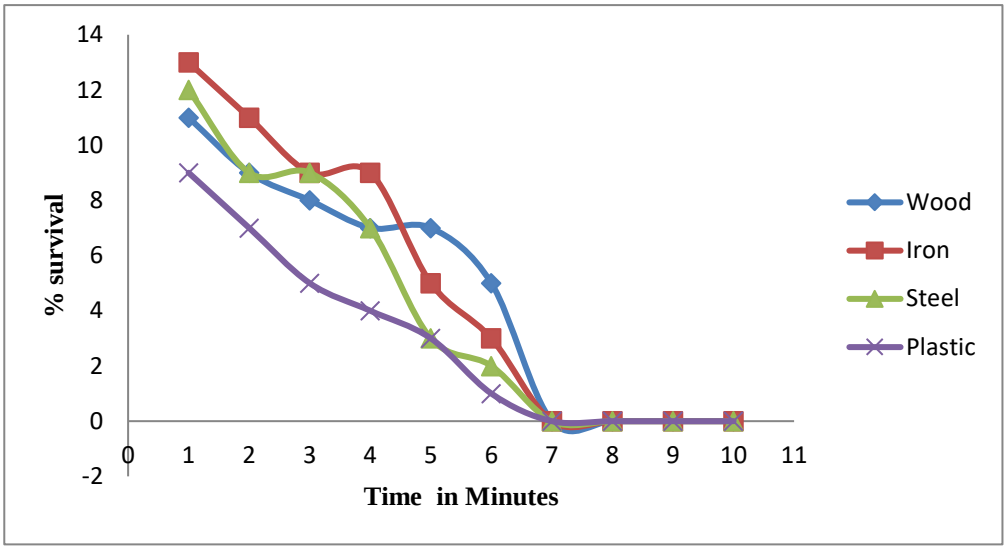

Figure Effect of leaf extract of *G. gummi-gutta* in controlling the growth of *S. typhi* on different cutting surfaces

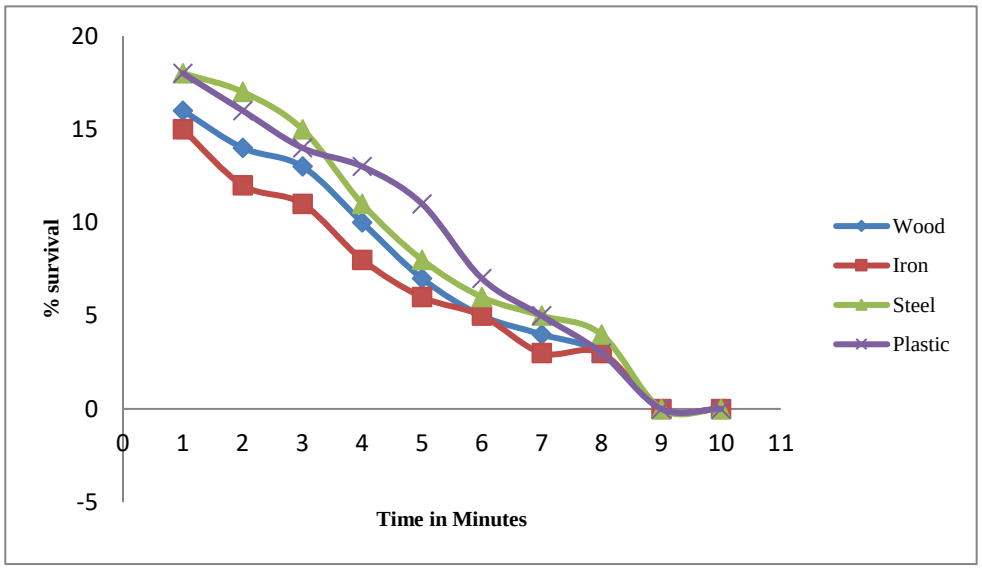

Figure 5. 6. Effect of leaf extract of *G. gummi-gutta* in controlling the growth of *B. subtilis* on different cutting surfaces

In controlling the growth of *B. subtilis* and *E. coli*, the time required was eight minutes (Figure 5.6 and 5.7).

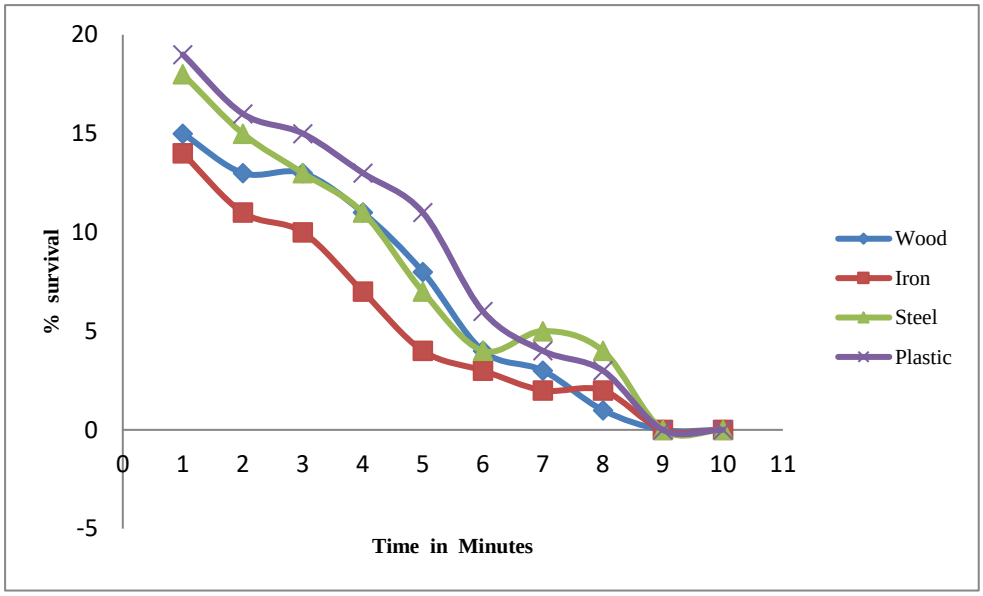

Figure 5. 7. Effect of Leaf extract of *G. gummi-gutta* in controlling the growth of *E. coli* on different cutting surfaces

In the case of *A. hydrophila*, the colonies were developed on tenth minute also (Figure 5.8), but number of colonies was very low. Fruit extract of *G. gummi-gutta* is found to be very effective in controlling the growth of *V. parahaemolyticus* and *S. typhi* within four minutes on different cutting surfaces and were depicted in the Figure 5. 9 and 5. 10.

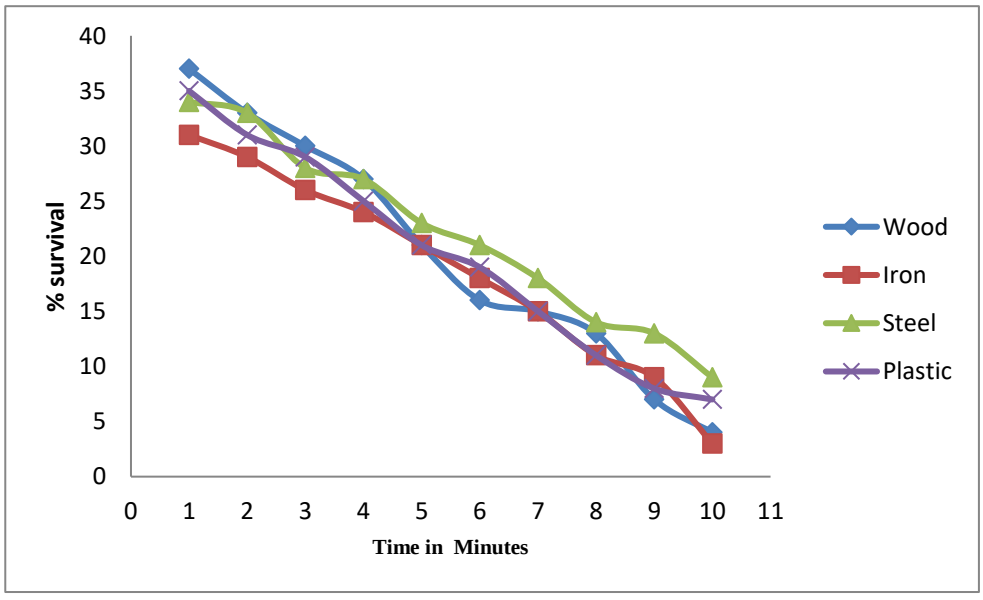

Figure 5. 8. Effect of leaf extract of *G. gummi-gutta* in controlling the growth of A. *hydrophila* on different cutting surfaces

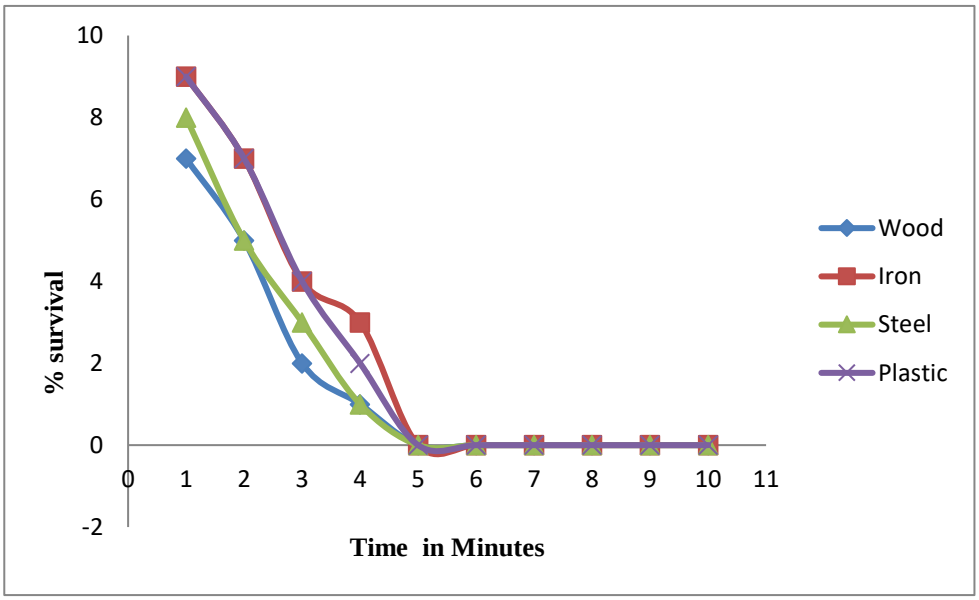

Figure 5. 9. Effect of fruit extract of *G. gummi-gutta* in controlling the growth of *V. paraheamolyticus* on different cutting surfaces

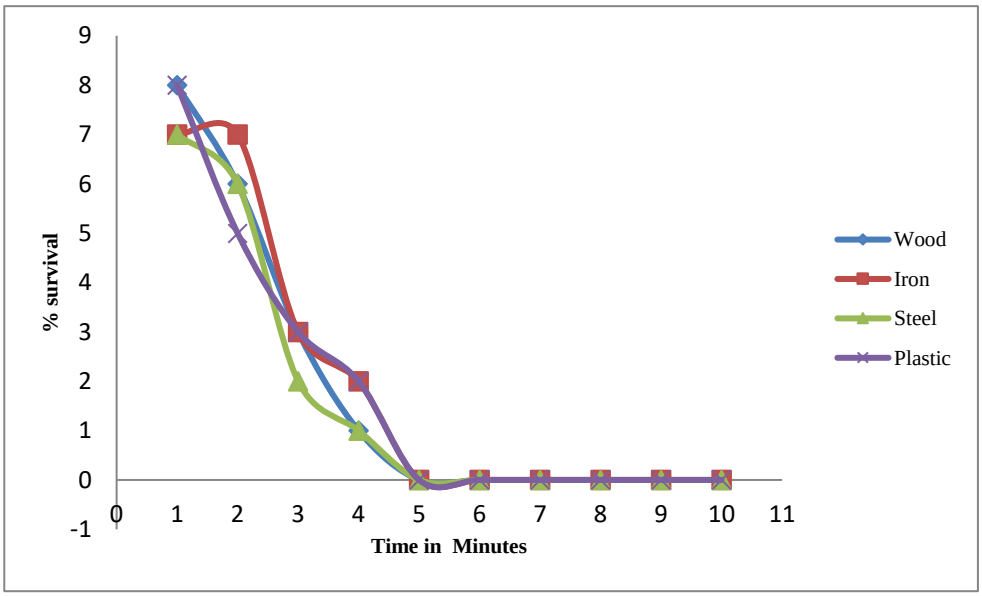

Figure 5. 10. Effect of fruit extract of *G. gummi-gutta* in controlling the growth of *S. typhi* on different cutting surfaces

B. subtilis growth was observed up to 6th minute (Figure 5. 11). Survival of *E. coli* was controlled in the eighth minute in iron surface, but on all other surfaces growth were recorded up to eighth minute (Figure 5. 12). The growth of *A. hydrophila* was in control at seventh minute (Figure 5. 13).

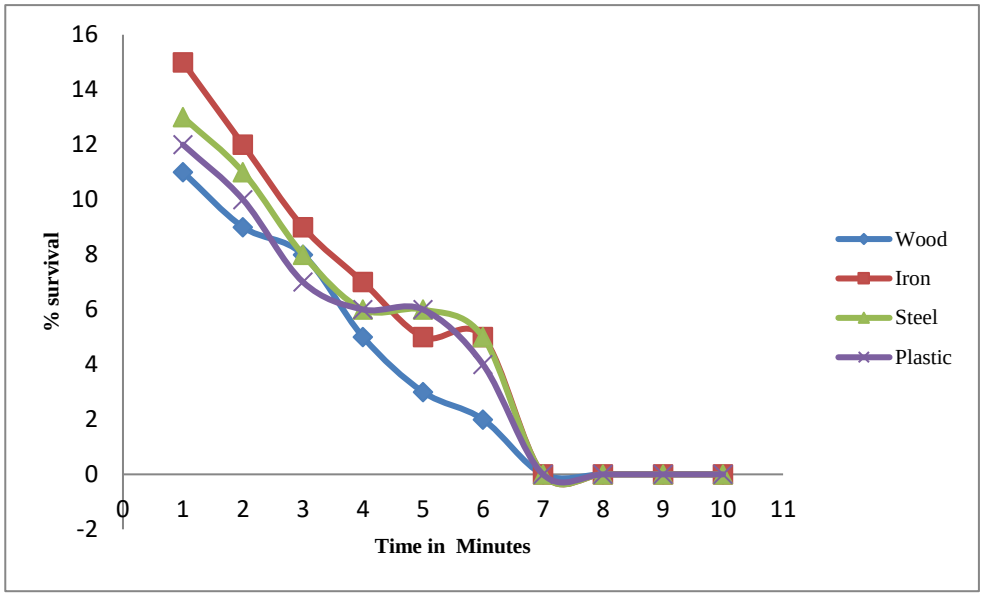

Figure. Effect of fruit extract of *G. gummi-gutta* in controlling the growth of *B. subtilis* on different cutting surfaces

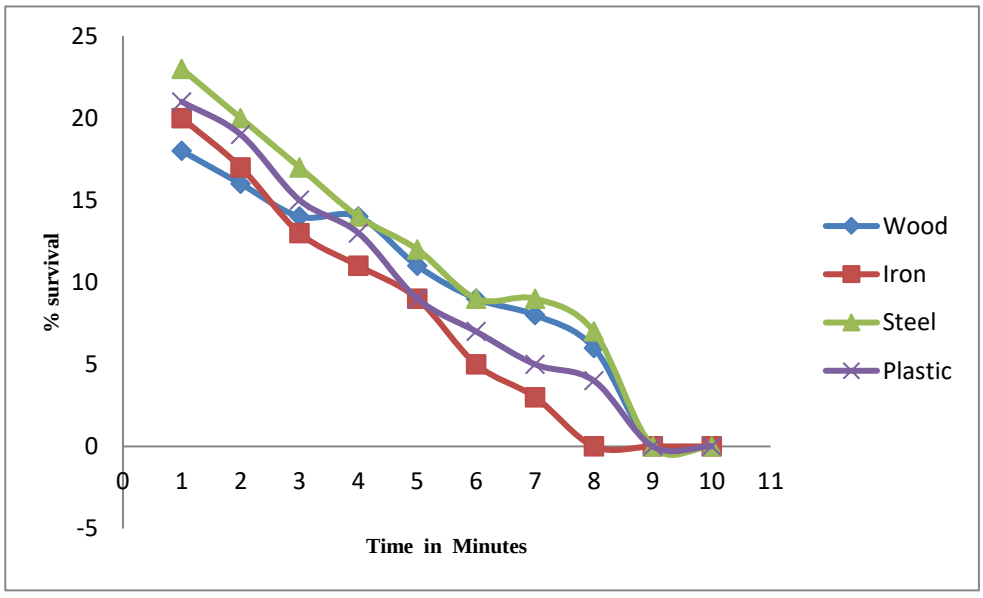

Figure. Effect of fruit extract of *G. gummi-gutta* in controlling the growth of *E. coli* on different cutting surfaces

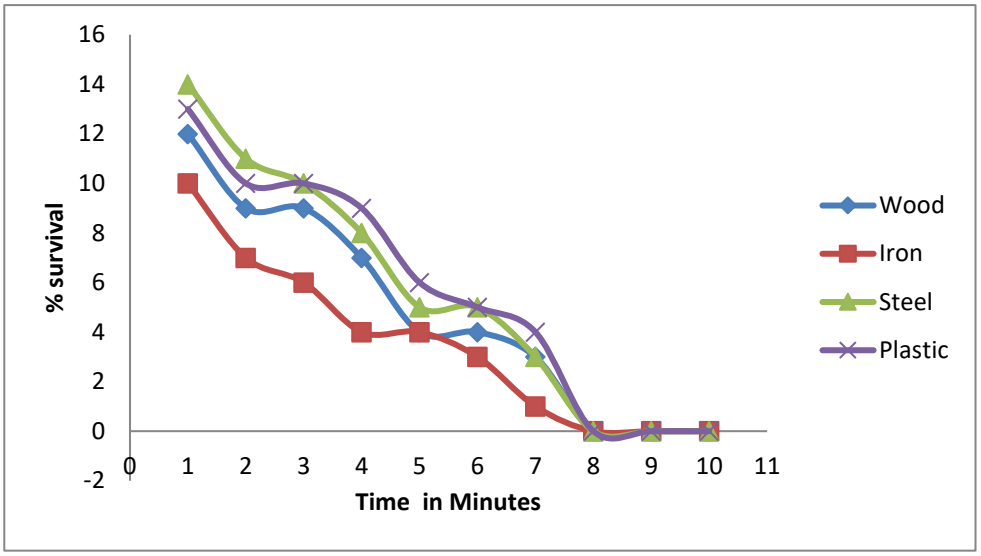

Figure. Effect of fruit extract of *G. gummi-gutta* in controlling the growth of *A. hydrophila* on different cutting surfaces

The effectiveness of *T. indica* leaf extract in controlling the growth of *V. paraheamolyticus* , *S. typhi, B. subtilis* and *E. coli* on different cutting surfaces was depicted in the Figure 5.14, 5.15, 5.16 and 5.17. The numbers of colonies were inactivated at every minute, but at the tenth minute also colonies were observed in all surfaces. But in the case of *A. hydrophila*, no colony were developed on wood and iron at the tenth minute (Figure 5. 18).

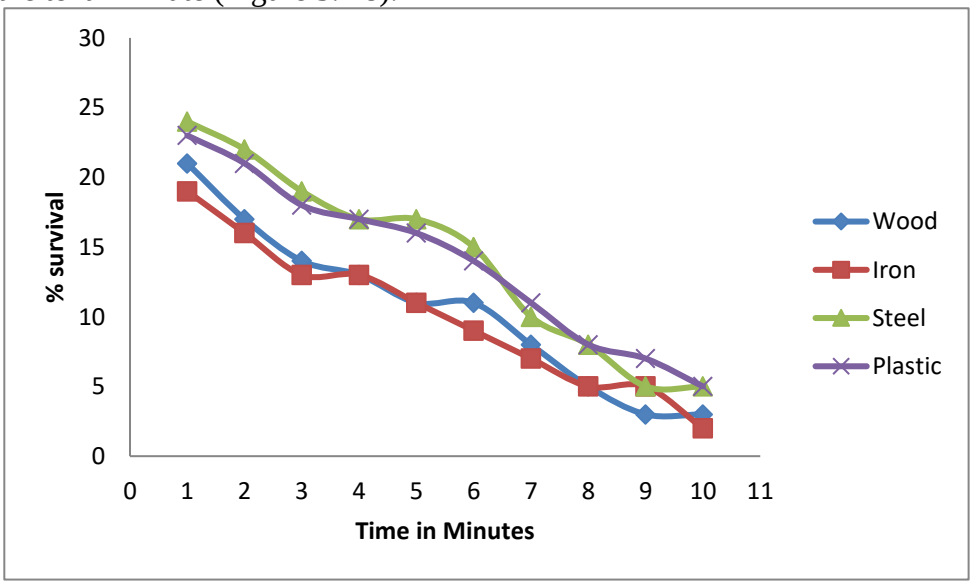

Figure. Effect of leaf extract of *T. indica* in controlling the growth of *V. parahaemolyticus* on different cutting surfaces

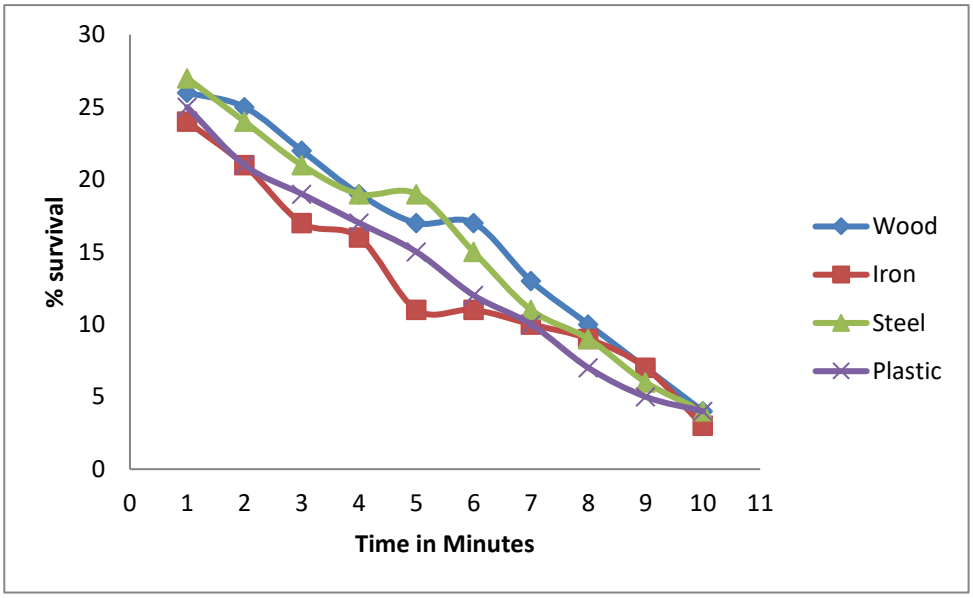

Figure. Effect of leaf extract of *T. indica* in controlling the growth of *S. typhi* on different cutting surfaces

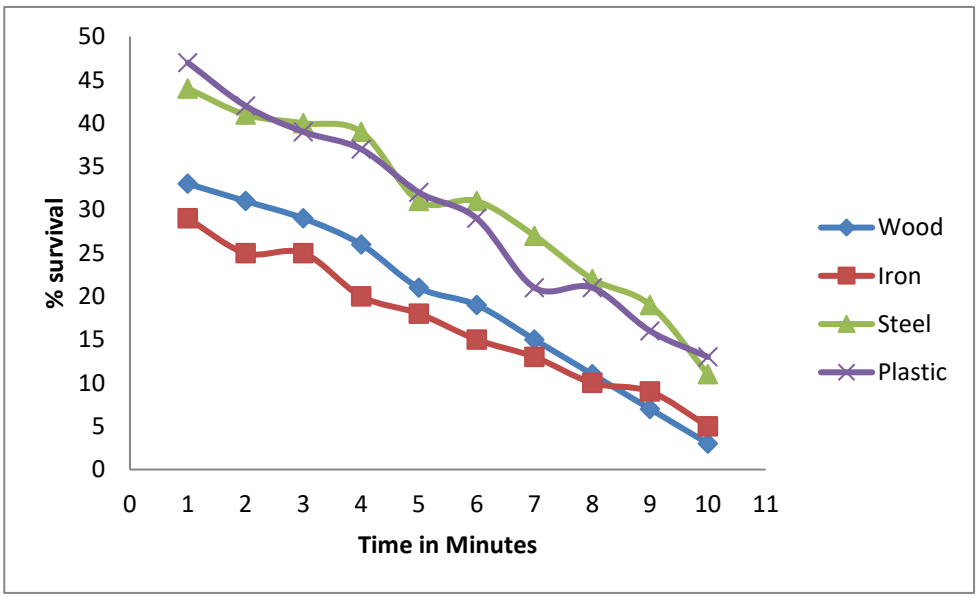

Figure. Effect of leaf extract of *T. indica* in controlling the growth of *B. subtilis* on different cutting surfaces

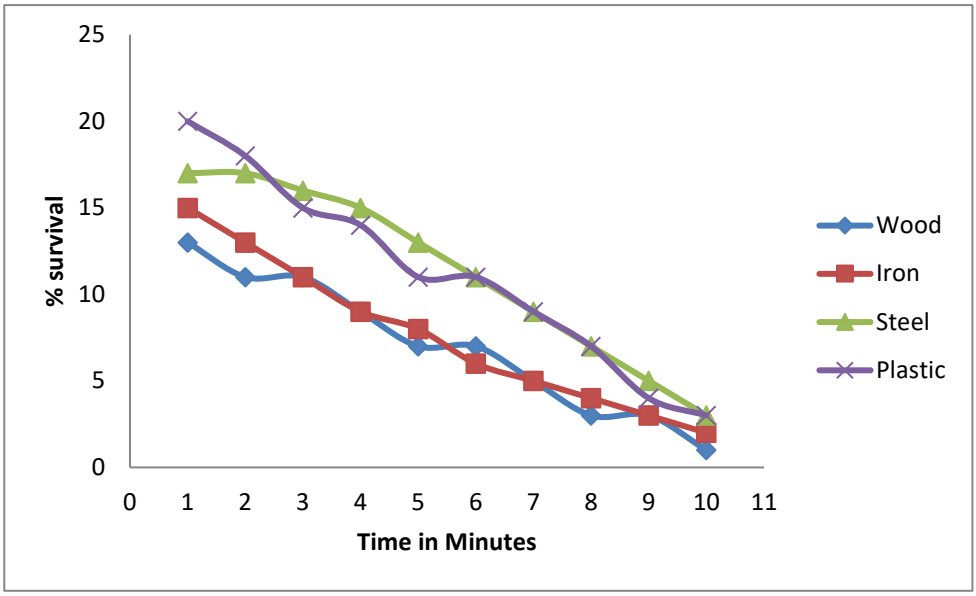

Figure: Effect of leaf extract of *T. indica* in controlling the growth of *E. coli* on different cutting surfaces

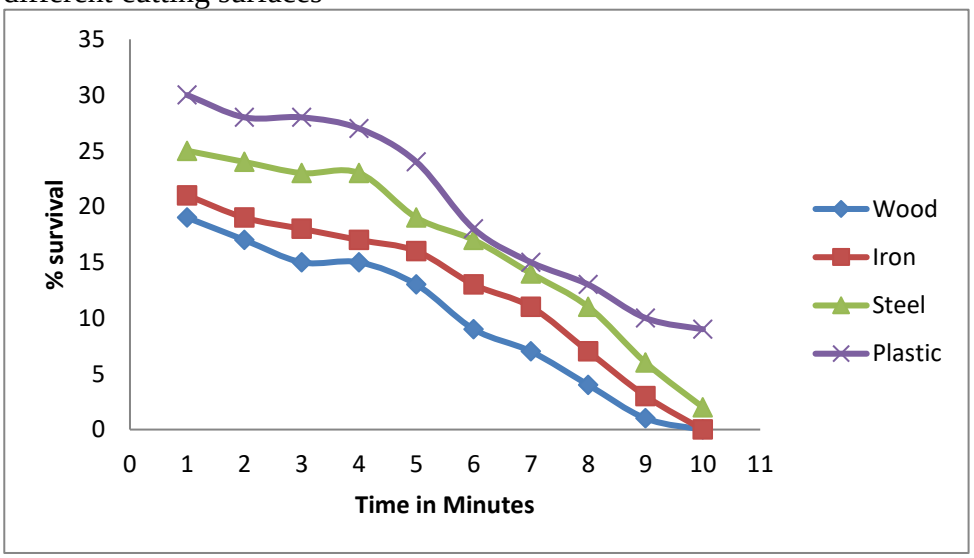

Figure Effect of leaf extract of *T. indica* in controlling the growth of *A. hydrophila* on different cutting surfaces

The fruit extract of *T. indica* in controlling the growth of *V. paraheamolyticus* on different cutting surfaces was depicted in the Figure 5. 19. Up to fourth minute the colonies were observed on all the test surfaces, after that no colonies were recorded. In controlling the growth of *S. typhi*, the extract was found to be effective at fifth minute on wood and plastic, but for iron and steel it was effective at sixth minute itself (Figure 5. 20).

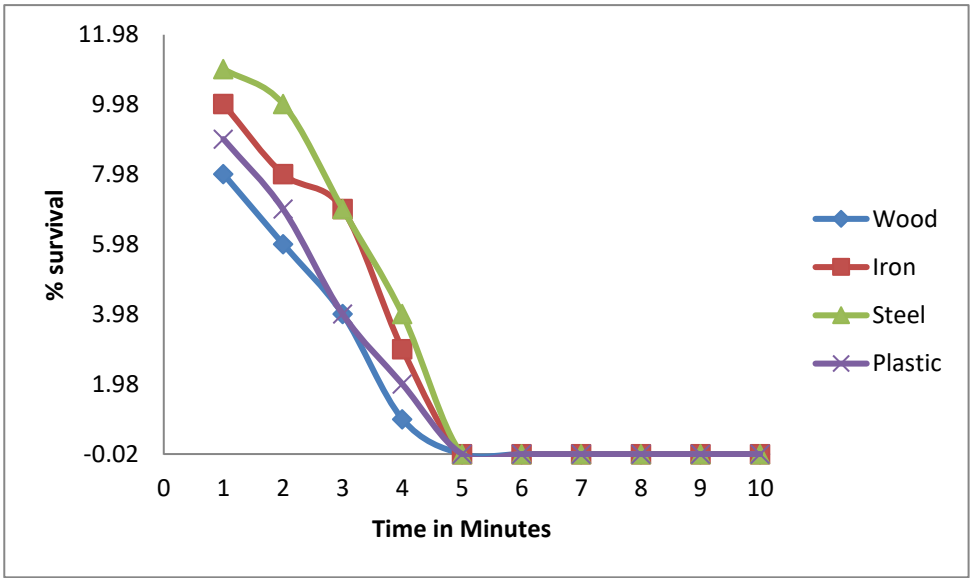

Figure Effect of fruit extract of *T. indica* in controlling the growth of *V. parahaemolyticus* on different cutting surfaces

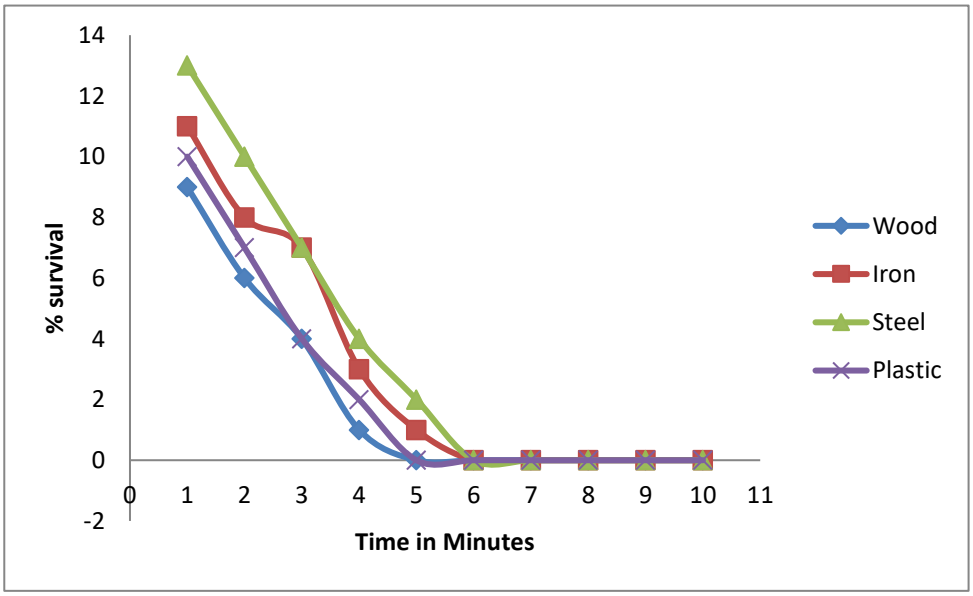

Figure Effect of fruit extract of *T. indica* in controlling the growth of *S. typhi* on different cutting surfaces

The survival of *B. subtilis* and *E. coli* were controlled by the fruit extract of *T. indica* at sixth minute (Figure 5. 21 and 5.22). In the case of *A. hydrophila*, no colonies were developed at fifth minute in wood and steel and in iron and plastic, colonies were controlled at sixth minute (Figure 5. 23).

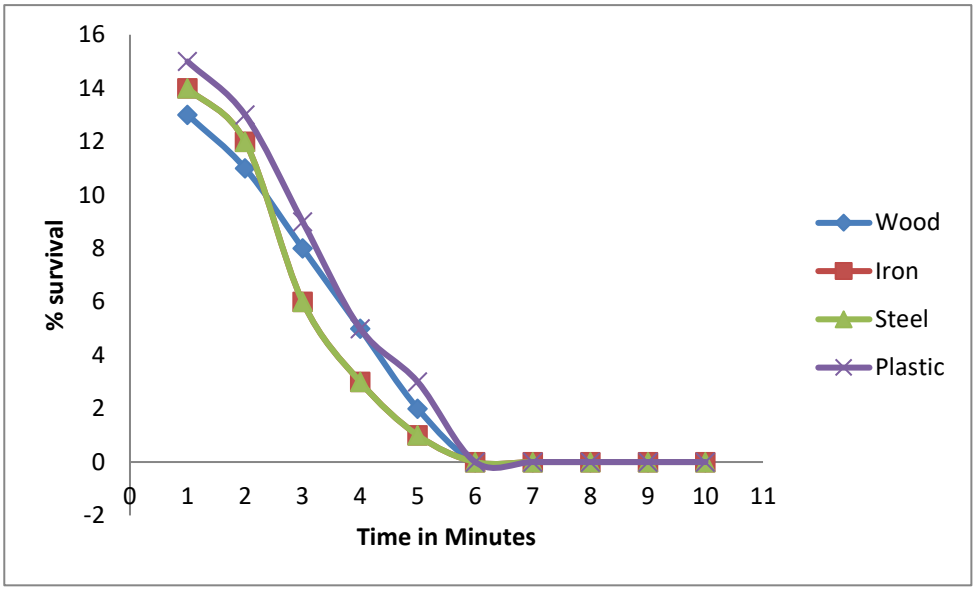

Figure Effect of Fruit extract of *T. indica* in controlling the growth of *B. subtilis* on different cutting surfaces

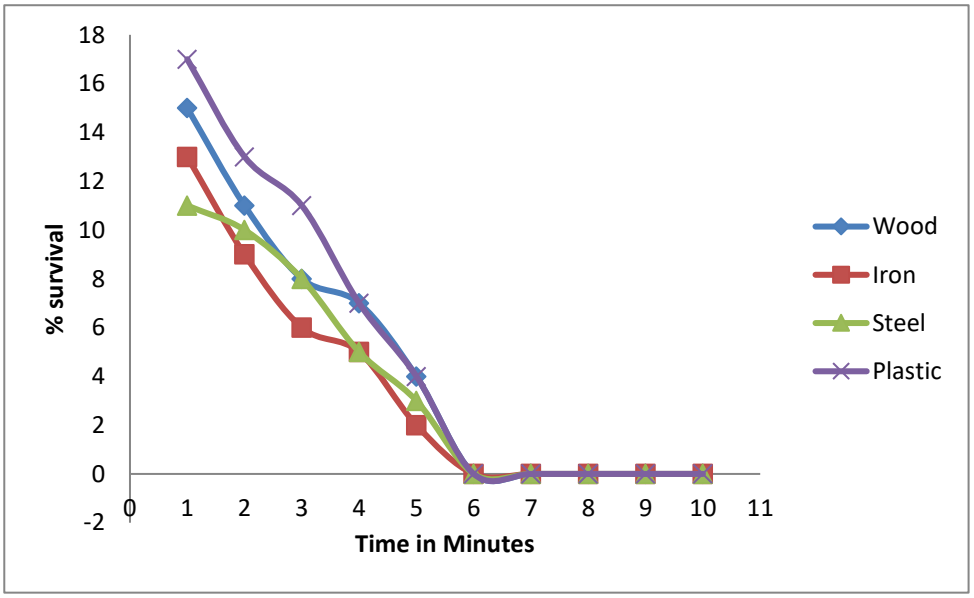

Figure Effect of fruit extract of *T. indica* in controlling the growth of *E. coli* on different cutting surfaces

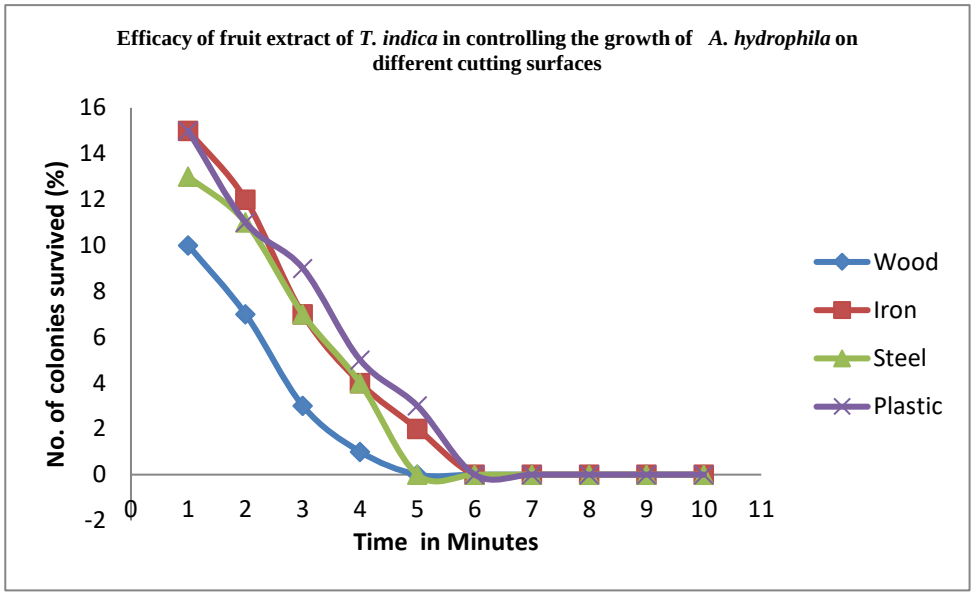

Figure Effect of fruit extract of *T. indica* in controlling the growth of *A. hydrophila* on different cutting surfaces

The growth of *V. parahaemolyticus* and *S. typhi* were controlled at sixth minute in wood and iron with the leaf extract of *M. oleifera*. But in steel and plastic the growth was observed at seventh minute (Figure 5. 24 and 5. 25). At eighth minute the growth of *B. subtilis* in steel and plastic was in control (Figure 5. 26). In wooden surface the growth was controlled at sixth minute and in iron it was at seventh minute. The colonies of *E. coli* were observed in all surfaces after a treatment of 10 minutes, but the percentage of survival have been decreased (Figure 5. 27). *A. hydrophila* survived on wood and iron at seventh minute and on steel and plastic at 8th minute (Figure 5. 28).

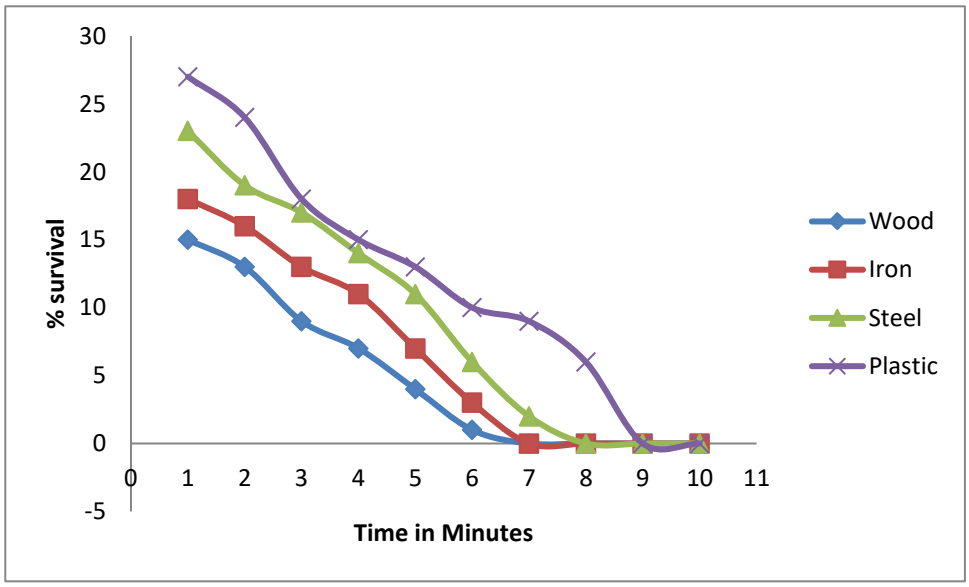

Figure Effect of leaf extract of *M. oleifera* in controlling the growth of

V. parahaemolyticus on different cutting surfaces

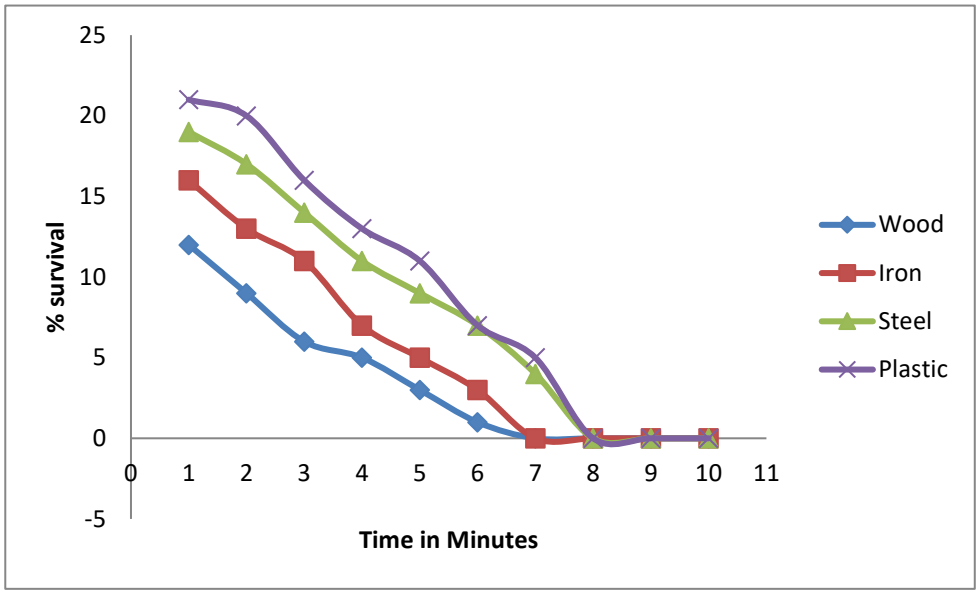

Figure Effect of Leaf extract of *M. oleifera* in controlling the growth of *S. typhi* on different cutting surfaces

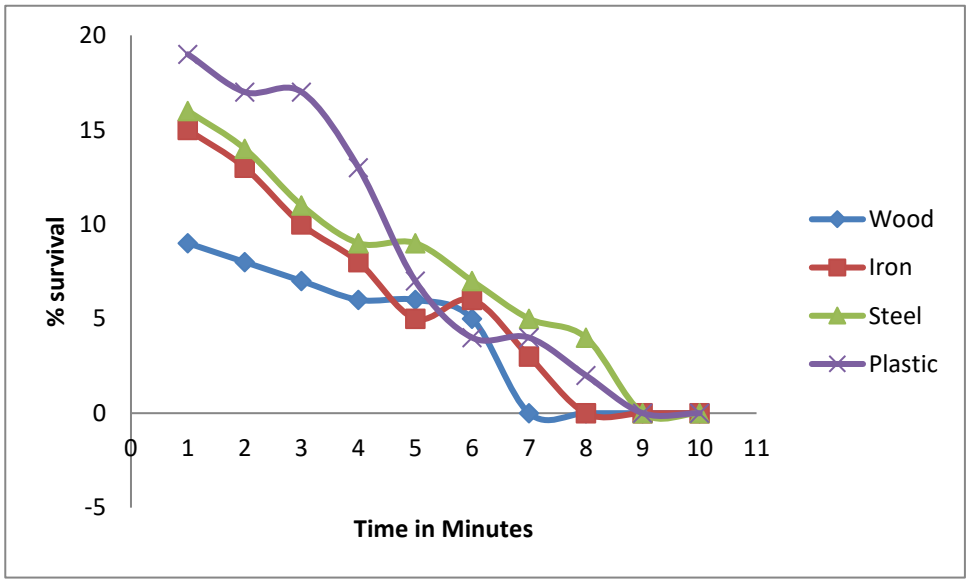

Figure Effect of Leaf extract of *M. oleifera* in controlling the growth of *B. subtilis* on different cutting surfaces

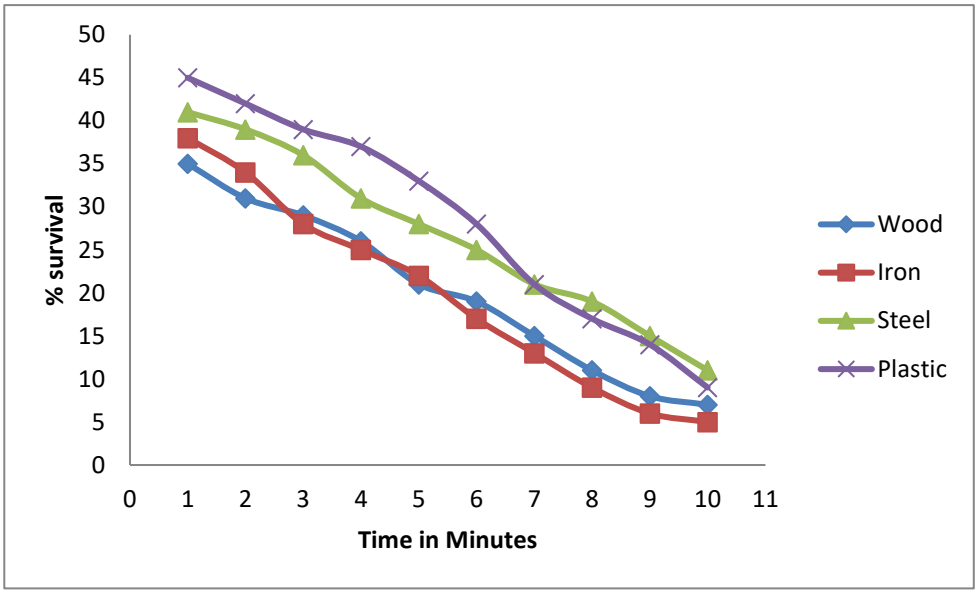

Figure Effect of Leaf extract of *M. oleifera* in controlling the growth of *E. coli* on different cutting surfaces

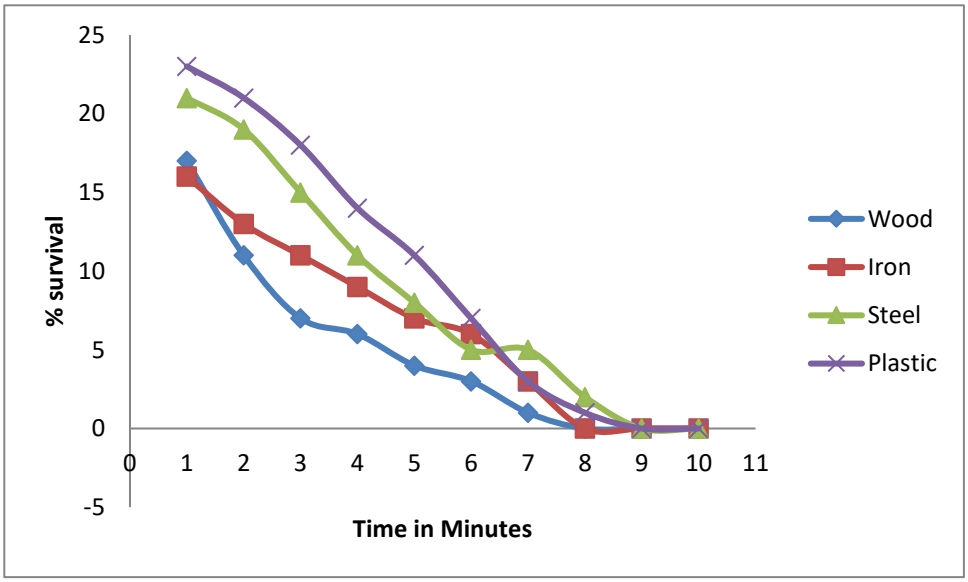

Figure 5. 28. Effect of Leaf extract of *M. oleifera* in controlling the growth of *A. hydrophila* on different cutting surfaces

Survival of the pathogens after the treatment of leaf extract of *G. gummi-gutta* on various meat cutting surfaces are represented in the Table 5.4. The cells of *S. typhi* was completely inactivated by the application of the extracts at seventh minute, followed by *V. parahaemolyticus* at eighth minute, *E. coli* and *B. subtilis* at ninth minute on all the studied surfaces and are represented in Table 5.5. However the activity of *G. gummi- gutta* fruit extract tackles the survival of *V. parahaemolyticus* and *S. typhi* within the fifth minute on iron surface. No surviving cell of *E. coli* was observed on iron at the eighth minute. In case of wood, steel and plastic the growth

was inactivated only at the ninth minute. *A. hydrophila* and *B. subtilis* cells were not observed on plastic and steel at seventh and eighth minute respectively.

The food borne pathogens were not controlled effectively with the application of the leaf extract of *T. indica*. But on wood and iron surface, *A. hydrophila* were completely eliminated after the tenth minute (Table 5.6). The pathogens may be controlled if the plant extracts were applied for some more minutes. *V. parahaemolyticus* was effectively controlled by the fruit extract of *T. indica* at fifth minute on all surfaces (Table 5.7). On plastic and wooden surface *S. typhi* cells were inactivated after fifth minute. On all test surfaces the survival of *E. coli* and *B. subtilis* were controlled in the sixth minute itself. No colonies of *A. hydrophila* were observed after fifth minute on steel and wood, and in sixth minute on iron and plastic.

The colonies of *E. coli* were not in control even after the application of leaf extract of *M. oleifera* on all surfaces. However the numbers of colonies were found to be in reduced (Table 5.8). *V. parahaemolyticus* and *S. typhi* were controlled on wood and iron surface at the seventh minute and at plastic and steel on eighth minute itself. On iron and plastic surface the survival of *A. hydrophila* and *B. subtilis* were controlled at the eighth minute.

There was no significant difference ($p < 0.05$) observed between the surfaces in controlling the growth of food borne pathogens after the application of the plant extracts.

Table Survival of the test pathogens after the treatment of leaf extract of *G. gummi-gutta* on various meat cutting surfaces at different time intervals

Test pathogens	**% survival after the treatment (Time in minutes)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
On wood										
V. paraheamolyticus	15	13	11	10	6	3	2	0	0	0
S. typhi	11	9	8	7	7	5	0	0	0	0
E. coli	15	13	13	11	8	4	3	1	0	0
A. hydrophila	37	33	30	27	21	16	15	13	7	4
B. subtilis	16	14	13	10	7	5	4	3	0	0
On iron										
V. paraheamolyticus	13	11	8	6	4	3	3	0	0	0
S. typhi	13	11	9	9	5	3	0	0	0	0
E. coli	14	11	10	7	4	3	2	2	0	0
A. hydrophila	31	29	26	24	21	18	15	11	9	3
B. subtilis	15	12	11	8	6	5	3	3	0	0
On steel										
V. paraheamolyticus	13	12	9	7	5	4	2	0	0	0
S. typhi	12	9	9	7	3	2	0	0	0	0
E. coli	18	15	13	11	7	4	5	4	0	0
A. hydrophila	34	33	28	27	23	21	18	14	13	9
B. subtilis	18	17	15	11	8	6	5	4	0	0
On plastic										
V. paraheamolyticus	16	13	11	9	5	3	2	0	0	0
S. typhi	9	7	5	4	3	1	0	0	0	0
E. coli	19	16	15	13	11	6	4	3	0	0

A. hydrophila	35	31	29	25	21	19	15	11	8	7
B. subtilis	18	16	14	13	11	7	5	3	0	0

V. paraheamolyticus- Vibrio paraheamolyticus, S. typhi – Salmonella typhi, E. coli -Escherichia coli, A. hydrophila – Aeromonas hydrophila, B. subtilis- Bacillus subtilis

Table Survival of the test pathogens after the treatment of fruit extract of *G. gummi-gutta* on various meat cutting surfaces at different time intervals

Test pathogens	**% survival after the treatment (Time in minutes)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
On wood										
V. paraheamolyticus	7	5	2	1	0	0	0	0	0	0
S. typhi	8	6	3	1	0	0	0	0	0	0
E. coli	18	16	14	14	11	9	8	6	0	0
A. hydrophila	12	9	9	7	4	4	3	0	0	0
B. subtilis	11	9	8	5	3	2	0	0	0	0
On iron										
V. paraheamolyticus	9	7	4	3	0	0	0	0	0	0
S. typhi	7	7	3	2	0	0	0	0	0	0
E. coli	20	17	13	11	9	5	3	0	0	0
A. hydrophila	10	7	6	4	4	3	1	0	0	0
B. subtilis	15	12	9	7	5	5	0	0	0	0
On steel										
V. paraheamolyticus	8	5	3	1	0	0	0	0	0	0
S. typhi	7	6	2	1	0	0	0	0	0	0
E. coli	23	20	17	14	12	9	9	7	0	0
A. hydrophila	14	11	10	8	5	5	3	0	0	0
B. subtilis	13	11	8	6	6	5	0	0	0	0
On plastic										
V. paraheamolyticus	9	7	4	2	0	0	0	0	0	0
S. typhi	8	5	3	2	0	0	0	0	0	0
E. coli	21	19	15	13	9	7	5	4	0	0
A. hydrophila	13	10	10	9	6	5	4	0	0	0
B. subtilis	12	10	7	6	6	4	0	0	0	0

V. paraheamolyticus- Vibrio paraheamolyticus, S. typhi – Salmonella typhi, E. coli -Escherichia coli, A. hydrophila – Aeromonas hydrophila, B. subtilis- Bacillus subtilis

Table Survival of the test pathogens after the treatment of leaf extract of *T. indica* on various meat cutting surfaces at different time intervals

Test pathogens	**% survival after the treatment (Time in minutes)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
On wood										
V. paraheamolyticus	21	17	14	13	11	11	8	5	3	3
S. typhi	26	25	22	19	17	17	13	10	7	4
E. coli	13	11	11	9	7	7	5	3	3	1

A. hydrophila	19	17	15	15	13	9	7	4	1	0
B. subtilis	33	31	29	26	21	19	15	11	7	3
On iron										
V. paraheamolyticus	19	16	13	13	11	9	7	5	5	2
S. typhi	24	21	17	16	11	11	10	9	7	3
E. coli	15	13	11	9	8	6	5	4	3	2
A. hydrophila	21	19	18	17	16	13	11	7	3	0
B. subtilis	29	25	25	20	18	15	13	10	9	5
On steel										
V. paraheamolyticus	24	22	19	17	17	15	10	8	5	5
S. typhi	27	24	21	19	19	15	11	9	6	4
E. coli	17	17	16	15	13	11	9	7	5	3
A. hydrophila	25	24	23	23	19	17	14	11	6	2
B. subtilis	44	41	40	39	31	31	27	22	19	11
On plastic										
V. paraheamolyticus	23	21	18	17	16	14	11	8	7	5
S. typhi	25	21	19	17	15	12	10	7	5	4
E. coli	20	18	15	14	11	11	9	7	4	3
A. hydrophila	30	28	28	27	24	18	15	13	10	9
B. subtilis	47	42	39	37	32	29	21	21	16	13

V. paraheamolyticus- Vibrio paraheamolyticus, S. typhi – Salmonella typhi,
E. coli -Escherichia coli, A. hydrophila – Aeromonas hydrophila,
B. subtilis- Bacillus subtilis

Table Survival of the test pathogens after the treatment of fruit extract of *T. indica* on various meat cutting surfaces at different time intervals

Test pathogens	**% survival after the treatment (Time in minutes)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
On wood										
V. paraheamolyticus	8	6	4	1	0	0	0	0	0	0
S. typhi	9	6	4	1	0	0	0	0	0	0
E. coli	15	11	8	7	4	0	0	0	0	0
A. hydrophila	10	7	3	1	0	0	0	0	0	0
B. subtilis	13	11	8	5	2	0	0	0	0	0
On iron										
V. paraheamolyticus	10	8	7	3	0	0	0	0	0	0
S. typhi	11	8	7	3	1	0	0	0	0	0
E. coli	13	9	6	5	2	0	0	0	0	0
A. hydrophila	15	12	7	4	2	0	0	0	0	0
B. subtilis	14	12	6	3	1	0	0	0	0	0
On steel										
V. paraheamolyticus	11	10	7	4	0	0	0	0	0	0
S. typhi	13	10	7	4	2	0	0	0	0	0
E. coli	11	10	8	5	3	0	0	0	0	0
A. hydrophila	13	11	7	4	0	0	0	0	0	0
B. subtilis	14	12	6	3	1	0	0	0	0	0
On plastic										
V. paraheamolyticus	9	7	4	2	0	0	0	0	0	0
S. typhi	10	7	4	2	0	0	0	0	0	0

E. coli	17	13	11	7	4	0	0	0	0	0
A. hydrophila	15	11	9	5	3	0	0	0	0	0
B. subtilis	15	13	9	5	3	0	0	0	0	0

V. paraheamolyticus- Vibrio paraheamolyticus, S. typhi – Salmonella typhi,
E. coli -Escherichia coli, Aeromonas hydrophila – A. hydrophila,
B. subtilis- Bacillus subtilis

Table Survival of the test pathogens after the treatment of leaf extract of *M. oleifera* on various meat cutting surfaces at different time intervals

Test pathogens	**% survival after the treatment (Time in minutes)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
On wood										
V. paraheamolyticus	15	13	9	7	4	1	0	0	0	0
S. typhi	12	9	6	5	3	1	0	0	0	0
E. coli	35	31	29	26	21	19	15	11	8	7
A. hydrophila	17	11	7	6	4	3	1	0	0	0
B. subtilis	9	8	7	6	6	5	0	0	0	0
On iron										
V. paraheamolyticus	18	16	13	11	7	3	0	0	0	0
S. typhi	16	13	11	7	5	3	0	0	0	0
E. coli	38	34	28	25	22	17	13	9	6	5
A. hydrophila	16	13	11	9	7	6	3	0	0	0
B. subtilis	15	13	10	8	5	6	3	0	0	0
On steel										
V. paraheamolyticus	23	19	17	14	11	6	2	0	0	0
S. typhi	19	17	14	11	9	7	4	0	0	0
E. coli	41	39	36	31	28	25	21	19	15	11
A. hydrophila	21	19	15	11	8	5	5	2	0	0
B. subtilis	16	14	11	9	9	7	5	4	0	0
On plastic										
V. paraheamolyticus	27	24	18	15	13	10	9	6	0	0
S. typhi	21	20	16	13	11	7	5	0	0	0
E. coli	45	42	39	37	33	28	21	17	14	9
A. hydrophila	23	21	18	14	11	7	3	1	0	0
B. subtilis	19	17	17	13	7	4	4	2	0	0

V. paraheamolyticus- Vibrio paraheamolyticus, S. typhi – Salmonella typhi,
E. coli -Escherichia coli, Aeromonas hydrophila – A. hydrophila,
B. subtilis- Bacillus subtilis

These results demonstrate that all the extracts have antibacterial efficacy, with a potency to disinfect the cutting surfaces in limited time. There was a significant effect was observed for the treatment assays with increased contact time and no significant differences were observed for different contact surfaces ($p< 0.05$).

With regards to the availability of these plants, the leaves were cheaply accessible as it is found everywhere in our local environs. Although, the fruit of *T. indica* and *G. gummi-gutta* are more or less expensive, lower concentrations of crude extracts were required to disinfect the cutting surfaces in limited time

compared to the leaves of *M. oleifera*, *T. indica* and *G. gummi-gutta*. However leaf extracts applied to the surfaces for 10- 15 minutes, could achieve better disinfection of the surfaces. Due to the porous nature of wood there is a chance to retain the bacterial colonies deep in the plant trunk cells. Even if we get no bacterial colonies after the 10^{th} minutes of disinfection, the extracts have to be kept for some more time so that these extracts can penetrate through these pores and can kill the pathogens residing deeper in the trunk. Generally in slaughter houses wooden planks were used as the platform for meat cutting and they daily uses synthetic disinfectants to clean the surfaces and once in week they remove the surface layer of wooden planks. If this platform were slash off daily up to 2-3 cm before applying these extracts, which may aid to remove the colonies residing deep in the trunks.

The study conducted by Prabhu *et al.* (2011), Gumgumjee *et al.* (2012), Elumalai *et al.* (2015) and Routhu *et al.* (2015) revealed the potential of *T. indica* fruit in controlling *E. coli* and *B. subtilis* which agrees with the present study. The antimicrobial activity of *T. indica* is attributed to the presence of compounds such as phenolics acids and flavonoids (Trila *et al.*, 2014). Shivakumar *et al.* (2013), Semwal *et al.* (2015), figure out the ethyl acetate, ethanol and hydro-alcoholic extracts from the fruit rind showed bactericidal activity against *E. coli* and *B. subtilis* which supports our findings. Peixoto *et al.* (2011) analysed the bactericidal activity of leaf extract and reported that *E. coli* and *Salmonella* were resistant. Conversely the findings of Oluduro *et al.* (2012) revealed that the leaf extracts do not control the growth of *E. coli*, *S. typhi* and *S. aureus* which contradict the present investigation. Torondell *et al.* (2014) reported that *E. coli* was resistant to the activity of leaf extract of *M. oleifera* which set with the present investigation.

Besides the environmental factors such as pH, water activity, temperature, atmospheric composition and initial microbial load of the food substrate, the efficacy of an antimicrobial compound depends on the type, genus, species, and strain of the target microorganism (Gould, 1989). Stratford *et al.*, 2003 reported that the chemical property determines the antimicrobial nature of a phytochemical compound. The properties include pKa value, hydrophobicity/ lipophilicity ratios, solubility, and volatility. The pH and polarity are the most prominent factors influencing the usefulness of a food antimicrobial (Davidson, 2001). For inhibiting the growth of microorganisms, use of combinations of antimicrobials were found to be more effective due to the reason that growth of some microbes was not controlled by the commonly used doses of antimicrobials (Beuchat, 2001 and Leistner, 1995).

The exact mechanism of antimicrobial activity of natural antimicrobials is not fully implicited, however, membrane disruption by terpenoids and phenolics, metal chelation by phenols and flavonoids, and effect on genetic material by coumarin and alkaloids are considered to hinder the survival of microorganisms (Cowan, 1999). The correct target(s) for natural antimicrobials are not well defined, as it is very complicated to identify a precise action site where many interacting reactions take place at the same time (Negi, 2012). Thymol, eugenol, and carvacrol which are the natural antimicrobials have been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of microorganisms (Burt, 2004). The

permeability of membrane were inhibited by carvacrol and thymol (Lambert *et al.,* 2001) which results in the breaking of phospholipid bilayer, by aligning between the fatty acid chain. Thymol binds to the membrane proteins hydrophobically and alters the permeability of membrane (Juven *et al.,* 1994). However the phenolic compounds exert their toxic effects at membrane level (Beltrame *et al.,* 1988) and the phenol changes the protein to lipid ratios of the membrane (Keweloh *et al.,* 1990). The respiration of several bacteria was inhibited by the activity of vanillin (Fitzgerald *et al.,* 2004) while terpenes disrupt the membrane integrity (Kararli *et al.,* 1995).

According to Lelieveld, *et al.,* (2003), a good disinfectant should have characteristics such as broad spectrum activity, environmental resistance (pH, water hardness, presence of organic matter), non- toxic, non-taint and ease to use. The antimicrobial mechanism of plant extracts, which affect microbial cells includes attacking the phospho lipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids (Arques *et al.,* 2008, Burt *et al.,* 2007, Skocibusic *et al.,* 2006). The plant extracts evaluated in this study, that satisfied these recommendations, can be act as an competent natural ecofriendly disinfectant emerging as a feasible substitute to control the food borne pathogens in the processing units. For using these in the food processing units, studies are required to eliminate the problem associated with odour of the plant extracts.

Conclusion

The discovery of natural products to control the pathogens and to guarantee safer food to the consumers is a challenge. Now a day the problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Even though industries have produced a number of new antibiotics and disinfectants, the resistance to microorganisms towards the antibiotics and disinfectants has increased drastically. The development of herb based products is essential as microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The resistance among bacteria towards these synthetic antibacterials is of serious concern. Thousands of biologically active molecules with antimicrobial properties were found in plants. The focus has to be on developing natural disinfectants with lower cost and higher antibacterial potency.

The present study evaluates the efficacy of selected plants extracts in controlling the survival of *Aeromonas hydrophila, Vibrio parahaemolyticus, Salmonella typhi, Escherichia coli* and *Bacillus subtilis* on wood, plastic iron and stainless steel surfaces. All the extracts were effective in inhibiting the growth of all strains assayed. Of which the fruit extract of *G. gummi-gutta* was found to have excellent potential in controlling the survival of all test pathogens on all surfaces at lower concentrations in limited time. So further studies have to be conducted for developing ecofriendly disinfectant from the fruit rind of *G. gummi-gutta*.

References

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