



## Research Article

### INVESTIGATION OF EFFECT OF NATURAL ANTIBACTERIAL AGENTS ON KITCHEN BORNE BACTERIA

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#### ABSTRACT

Health and hygiene are the primary requirements for human beings to live comfortably and work with maximum efficiency. The study conducted focuses preliminarily on the development of herbal finishes for conventional cotton terry fabrics used in kitchens to suppress the kitchen borne bacteria. An investigation was undertaken to determine the bacterial contamination using 6 samples from vegetarian and non vegetarian kitchens of common houses and hotels were collected and inferred presence of bacterial species namely *Moraxella sp*, *Staphylococcus sp*, *Escherichia sp*, *Pseudomonas sp*, *Klebsiella sp*, *Enterobacter cloacae*, *Klebsiella pneumonia*. Natural antimicrobial plants *Syzygium aromaticum* (Ginger) and *Zingiber officinale* (Clove) were selected, and their bioactive compounds were extracted by Soxhlet and applied in different proportions on 100% white cotton terry fabrics by using pad-dry-cure method. Five samples were produced in different proportions of Ginger and Clove. SEM and FTIR investigations clearly ensure the presence of the herbal in the fabrics. The antibacterial activity of the fabrics were assessed using standard AATCC 147 test methods and the inhibition zone ranges from 1 mm to 7 mm. The ginger 25% clove 75% sample had better antibacterial activity against all the bacteria when compared to the other samples. The finish is also evaluated by real time usage.

**Keywords:** Kitchens, Bacteria, Clove, Ginger, Real Time, Hygiene

#### INTRODUCTION

The textile and clothing industry normally seen as “traditional industry” is an important part of the European and Asian manufacturing industry. To protect the mankind and to avoid cross contamination, a special finish like antimicrobial finish<sup>1</sup> has become increasingly necessary. A fabric in kitchen has become important all over the world and a considerable usage is going in this direction. The usage of fabric in kitchen is to free kitchen wears from wetness, which makes them easily get contaminated and pave way for spoiling the food.

Presumably, food-borne diseases sometimes acquired in hotels and restaurants are through dishes, plates and other kitchen equipments<sup>2</sup>. It is found that 74% cloths used in cleaning dishes and cutting equipment surfaces were contaminated with one or more of the following organisms *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Clostridium perfringens*<sup>3-5</sup>. It is clear that wiping kitchen equipment with cloths may result in the contamination of equipment. It is reported that microbial cells attached to equipment surfaces, especially those that come in contact with the food, may not be easily killed by chemical sanitizers or heat designed to be effective against unattached microbial cells; and thus they can contaminate food<sup>6,7</sup>. Surface and equipment used in kitchen may look sparkling clean, yet bacteria may be present in large numbers<sup>8,9</sup>. The intention of food safety is to prevent food poisoning, (the transmission of disease through food) and to maintain the wholesomeness of the food product though all

stages of processing, until it is finally served. Therefore, one important task is to use hygienic products in kitchen<sup>7</sup>.

Cotton fabrics, by virtue of their absorption characteristics and proximity to human body, provide an excellent medium for the adherence, transfer and propagation of infection<sup>10</sup> causing microbial species, especially in cases where work wears are worn, in places such as hospitals, clinics, food industry, kitchens clothes, etc. The presence and growth of these microorganisms can cause health problems, odors and finally fabric deterioration. In the last few decades, synthetic antimicrobial agents are effective against microbes but they are a cause of concern due to the associated side-effects, action on non-target microorganisms and water pollution<sup>1</sup>. Hence, there is a great demand for antimicrobial textiles based on eco-friendly natural agents which not only help to reduce effectively the ill effects associated due to microbial growth on textile material but also comply with the statutory requirements imposed by regulating agencies<sup>11,12</sup>. Various natural biocides from natural plant are extracted and used as antimicrobial finishing for textiles play an ever growing role for bactericidal and fungicidal properties<sup>13-18</sup>.

Ginger<sup>19-24</sup> has been used for treating cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, less of appetite, and rheumatism. It also has good antimicrobial properties. The properties of clove<sup>25-27</sup> include antioxidant, insecticidal, antifungal and antibacterial properties. By tradition, it has been used in food preservation as flavoring and antimicrobial sub-stance. Hence, the herbs *Syzygium*

*aromaticum* (Ginger) and *Zingiber officinale* (Clove) were used in this study to suppress kitchen borne bacteria by finishing on Cotton fabric through pad-dry-cure.

## MATERIALS AND METHODS

### Materials

Cotton Fabrics with Terry Pile Structure were used for this study. The terry pile structure absorbs more amount of water, mostly preferred in Kitchens to clean spills, dry plates, wipe the moisture on surface, etc. Hence for dish cloths, they are appropriate material and hence used for experiment purpose in this study. Terry cotton was procured from the local market in the southern part of India. The specifications of the dish cloths purchased were white colour, Size of 25 cm x 25 cm (square fabric). Herbs namely Clove and Ginger were procured from local markets in Coimbatore, Tamilnadu, India from Indian Medicines approved Retail Outlets were used for finishing on cotton terry towels to evaluate for antibacterial activity.

### Sample Size and Specification

A total of 6 samples of kitchen cloths were provided to several kitchens and asked them to use it for 20 days which is washed 5 days once. Among 6 samples 2 samples were provided to vegetarian and non vegetarian kitchens of houses, 1 sample to vegetarian house kitchen, 1 to non vegetarian hotel kitchens, 1 to vegetarian hotel kitchen and 1 to vegetarian and non vegetarian hotel kitchens. The samples were named as A1, A2, A3, A4, A5 and A6. These samples were provided to the kitchens of different places can be observed in Table 1.

### Isolation of Bacteria from Kitchen used Cotton Terry Fabric Sample

A Quarter-Strength Ringer solution was used for extracting the bacteria from fabric. A conical flask containing 20ml of quarter-strength Ringer solution was taken and a fabric of area 25cm<sup>2</sup> was put inside the conical flask. The flask is cotton plugged and then they were incubated in orbital shaker for 30minutes at 37°C with 120-130 rpm. The solution is then used for dilution and plating on Petri plates. For isolation of bacteria, test tubes each of 9ml distilled water were taken and cotton plugged and was sterilized in a cooker of 121°C for 20minutes. 1ml of fabric mixed quarter-strength Ringer solution was taken and poured on 9ml test tube for dilution. Then 1ml from that test tube was taken and poured it on another 9ml test tube. Likewise the dilutions on number of test tubes were made for dilution purpose and also to get individual colonies on Petri plates using serial plating technique. For plating nutrient medium agar used and steps are followed as per standard procedures<sup>6</sup>.

### Morphological and Confirmatory Biochemical Parameters of Isolates

The various physiological and biochemical tests were carried out according to methods outlined in the Bergey's Manual of Determinative Bacteriology.

### Colony morphology

The isolates were streaked on nutrient agar medium, incubated at 37°C for 48 h and observed for the colony morphology: Gram staining, Capsular staining, Motility test, Indole production, Methyl Red and Voges, Proskauer (MR-VP) test, Citrate utilization test, Triple sugar iron (TSI) test, Oxidase test, Catalase test, Urea hydrolysis tests are followed as per standard Microbiological test steps and procedures followed to determine

the exact bacteria genus isolated from the used cotton terry dish clothes.<sup>6</sup>

### Extraction from Clove and Ginger

*Syzygium aromaticum* (Ginger) and *Zingiber officinale* (Clove) were chosen and used for the experimental purpose. The root of Ginger plant was chosen for extraction since it has higher antibacterial activity when compared to the other parts. It is sun dried for seven days and ground into fine powder using an electric grinder. 20 grams of ginger powder is mixed in 100ml of 95% ethanol and it is extracted at 78°C using soxhlet apparatus. The mixture was stirred, covered, and allowed to stand for 24hrs, and filtered using sterile Whitman No.1 filter paper. The filtrate was concentrated to 20ml on a water bath and evaporated to dryness at room temperature. In case of Clove, the flower buds of clove plant were sun dried for seven days and ground into fine powder using an electric grinder. Same procedures used for Ginger were then followed to acquire extract from Clove<sup>23</sup>. The extracts were then dried further to get crude form of extracts using vacuum desiccator.

### Fabric Treatment with Herbal by Direct application Method

Bleached Cotton Terry fabric was padded with 5% aqueous herbal extract by using pad-dry-cure technique. Drying was carried out at 80°C for 5 min and curing was carried out at 150°C for 3 min. The samples were dried at ambient temperature without washing. Five samples were produced in different proportions of Ginger and Clove. They were named as S1, S2, S3, S4 and S5 namely in the proportion:100% Ginger, 75% Ginger and 25% Clove, 50% Ginger and 50% Clove, 25% Ginger and 75% Clove and 100% Clove respectively were summarized in Table 3.

### Investigations of Treated Cotton Fabric Samples

Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) were done for Cotton Terry Treated and Untreated fabrics. The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. SEM images of the treated and untreated samples were obtained. FTIR Analysis gives the fact that bonds and functional groups vibrate at characteristic frequencies. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks. From the FTIR images the presence of the functional groups of both the herbals can be identified to prove that the herbal trace is present on the fabric.

### Drug Release Test through UV Spectroscopy

Drug release tests were completed by ordinary disintegration systems prescribed for single - substance items, utilizing basket container (USP Apparatus 1) at 75 and 100 rpm. Examining aliquots of 5.0 ml were pulled back at 0, 5, 10, 15, 30 and a hour, and supplanted with an equivalent volume of the crisp medium to keep up a steady aggregate volume. After the finish of each test time, tests aliquots were separated, weakened in disintegration medium, when vital, and measured. The test of the three tried items was performed utilizing beforehand approved spectrophotometric strategy, and the substance comes about were utilized to compute the rate discharge on each season of disintegration profile. The UV spectrophotometric technique used to dissect the DAR tests in phosphate cushion pH 7.0 with 0.75 % of sodium lauryl sulfate (SLS) disintegration medium

was approved for specificity, linearity, exactness and precision, as per USP Pharmacopeia and ICH rule. All absorbance were resolved at 460 nm.

**Antimicrobial Activity Determination**

Testing for antibacterial activity of treated was carried out using a qualitative method as per the standard AATCC test method 147 (Disc diffusion method). Treated samples were estimated for their antibacterial activity by positioning them on nutrient rich medium where high rate of growth of bacteria occurs.

**Real time testing**

The herbal treated terry cotton fabric was again given to vegetarian and non vegetarian kitchens of houses and hotels and asked them to use it for 10 days. The fabric was washed twice at an interval of 5 days. Again these fabrics tested for bacterial contamination. By this test, the efficiency of finish agents can be well appreciated. Two samples finished with the proportion of 50% Ginger and 50% Clove, 100% Clove were used for testing, since it has the higher antibacterial property when compared to the other proportion of concentrations.

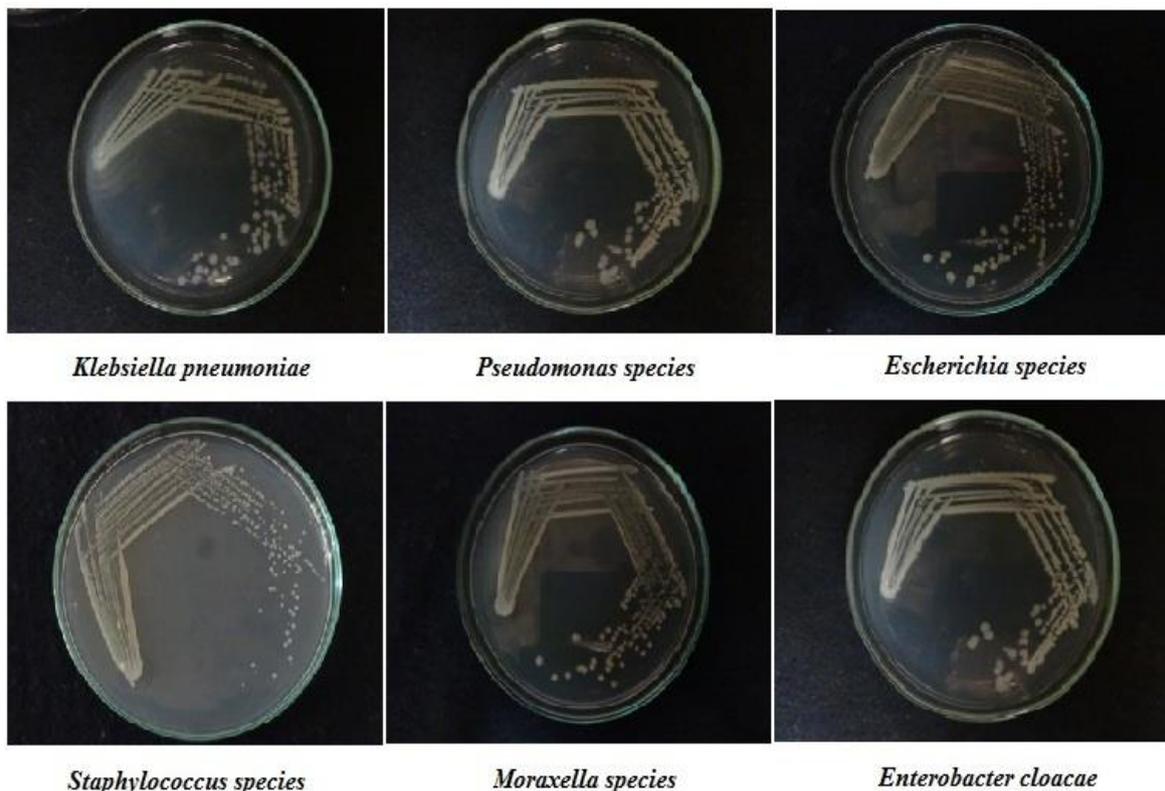


Figure 1: Bacteria colonies isolated from kitchen dish clothes

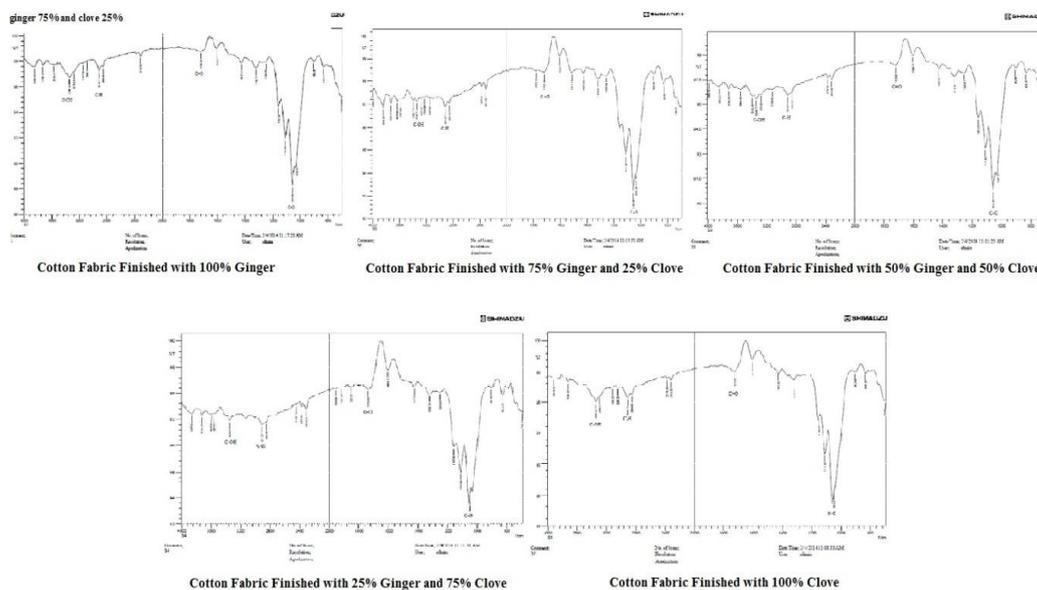


Figure 2: FTIR of cotton fabric

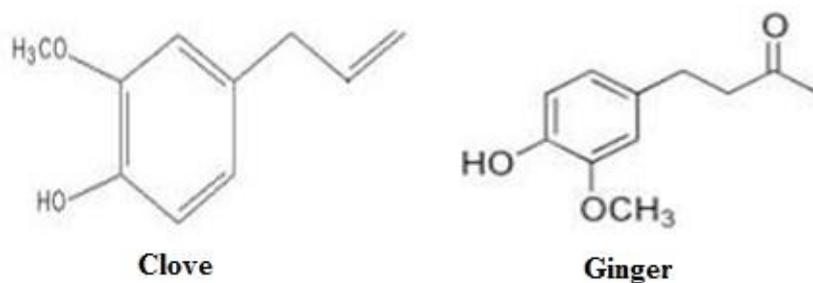


Figure 3: Chemical structure of clove and ginger powder

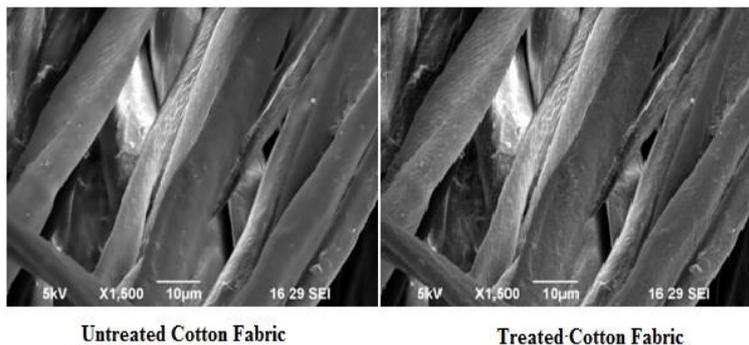


Figure 4: SEM images of cotton fabric

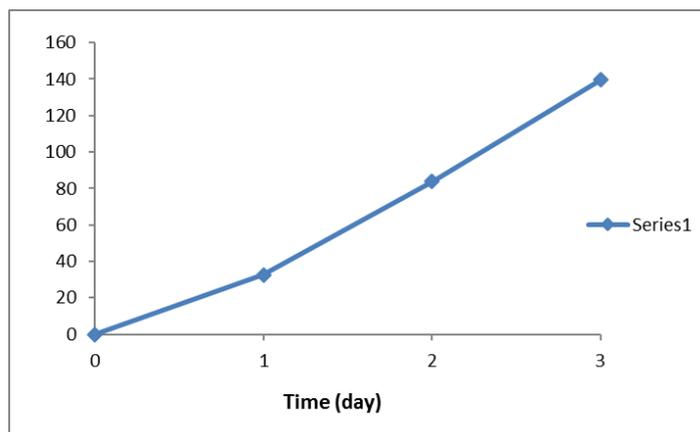


Figure 5: Drug Release test



Higher Zone of Inhibition (Z.O.I.) among samples were shown Sequentially Starting from S1 on Left Side till S6 on Right Side



Lower Zone of Inhibition (Z.O.I.) among samples were shown Sequentially Starting from S1 on Left Side till S6 on Right Side

Figure 6: Zone of inhibition for natural extract treated cotton fabric samples

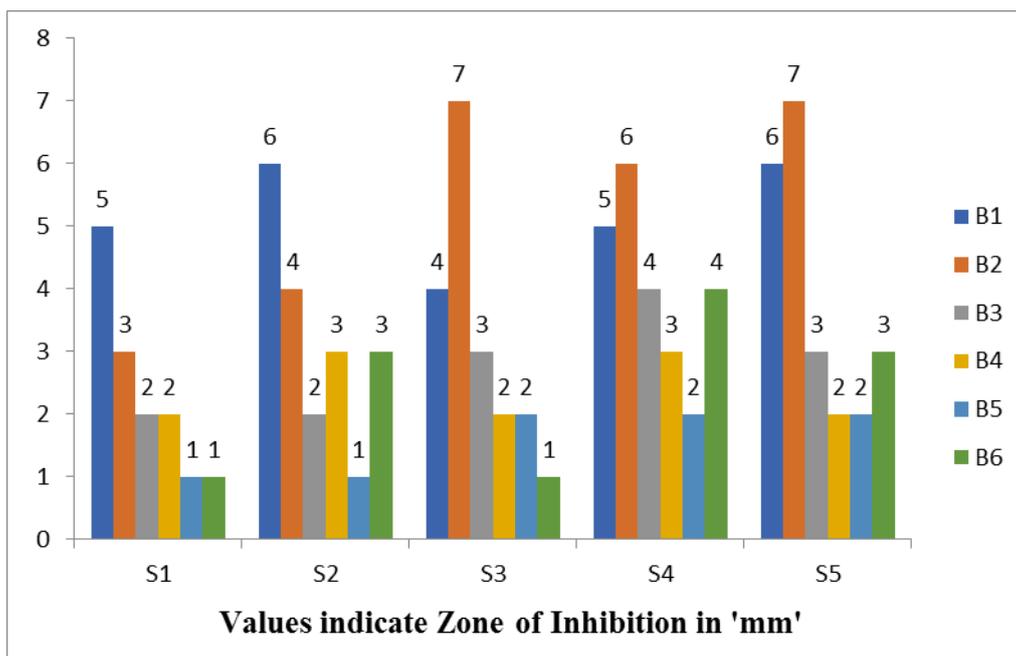


Figure 7: Antibacterial efficiency of herbal treated cotton fabric samples

Table 1: Isolation of bacteria from kitchen used samples

Sample Code	Sample specification	Types of Bacteria colonies isolated in the used kitchen samples					
		B1 <i>Moraxella species</i>	B2 <i>Staphylococcus species</i>	B3 <i>Escherichia species</i>	B4 <i>Pseudomonas species</i>	B5 <i>Enterobacter cloacae</i>	B6 <i>Klebsiella pneumoniae</i>
A1	Vegetarian Houses	a	X	X	a	a	a
A2	Vegetarian Houses	a	a	a	a	a	X
A3	Non vegetarian Houses	a		X	a	X	a
A4	Vegetarian Hotels	a	a		a	X	a
A5	Non vegetarian Hotels	X	X	X	X	X	a
A6	Vegetarian and Non vegetarian Hotels	a	a	a	a	a	X

X – Presence of Bacteria, a – Absence of Bacteria

Table 2: Drug release test results

Time (day)	OD at 426nm	µg/ml	Herbal drug release
0	0	0	0
1	0.823	42.50	31.30
2	0.932	49.26	82.46
3	1.235	57.43	113.3

Table 3: Antibacterial efficiency (Z.O.I) test results

Sample Code	Sample Specification	B1	B2	B3	B4	B5	B6
S1	Ginger 100%	5 mm	3 mm	2 mm	2 mm	1 mm	1 mm
S2	Ginger 75% and Clove 25%	6 mm	4 mm	2 mm	3 mm	1 mm	3 mm
S3	Ginger 50% and Clove 50%	4 mm	7 mm	3 mm	2 mm	2 mm	1 mm
S4	Ginger 25% and Clove 75%	5 mm	6 mm	4 mm	3 mm	2 mm	4 mm
S5	Clove 100%	6 mm	7 mm	3 mm	2 mm	2 mm	3 mm

Table 4: One way ANOVA for antimicrobial testing results

		Sum of Squares	Degree of Freedom	Mean Square	F- Value	Significant Value
S2	Between Groups	12.333	3	4.111	3.289	0.242
	Within Groups	2.500	2	1.250		
	Total	14.833	5			
S3	Between Groups	21.833	3	7.278	14.556	0.065
	Within Groups	1.000	2	0.500		
	Total	22.833	5			
S4	Between Groups	7.500	3	2.500	2.000	0.350
	Within Groups	2.500	2	1.250		
	Total	10.000	5			
S5	Between Groups	21.833	3	7.278	14.556	0.065
	Within Groups	1.000	2	0.500		
	Total	22.833	5			

Table 5: Bacteria isolation for natural extract treated cotton fabric

S. No.	Proportion of Samples	Result
1.	Clove 75% and ginger 25%	No bacterial contamination
2.	Clove 75% and ginger 25%	No bacterial contamination

## RESULTS AND DISCUSSION

### Bacteria Isolated and Identified from Used Kitchen Dish Cloths

The bacteria present in the dish cloths were identified by using diffusion plating technique followed by staining and then lastly biochemical Confirmatory tests. Six bacteria as shown in Figure 1 are identified from the used dish cloths. They are coded as in Table 1 and are summarized as B1, B2, B3, B4, B5 and B6. Each code representation: B1 - *Moraxella species*; B2 - *Staphylococcus species*; B3 - *Escherichia species*; B4 - *Pseudomonas species*; B5 - *Enterobacter cloacae* and B6 - *Klebsiella pneumoniae*.

### Fourier Transform Infrared Spectroscopy (FTIR) Results

FTIR images from Figure 2 infers the presence of the functional groups of both the herbals "Clove and Ginger" on Treated Cotton Terry Fabric and can be well understand from the wavelength ranges: 3600-3200  $\text{cm}^{-1}$  shows C-OH extensions and 1150-1050  $\text{cm}^{-1}$  of C-O extensions indicate the presence of Clove on Treated Fabric, while wavelength 2960-2850  $\text{cm}^{-1}$  belongs to C-H extensions and 1820-1620  $\text{cm}^{-1}$  C=O of carboxylic confirms the Glove extract key components on Treated Cotton Fabric. FTIR results proved that the herbals were deposited on the treated fabric. These functional groups were present in the corresponding chemical structure of Clove and Ginger respectively. Chemical Structure can be referred from the Figure 3.

### Scanning Electron Microscopy (SEM) Inference

Figure 4 shows SEM images obtained for Untreated and Treated Cotton Fabric Samples, on comparison of the SEM images at magnification level of 10  $\mu\text{m}$  range (enlarged image) infers the presence of herbal agents' key component in particles form are deposition on the Treated Cotton fabric surface while no particle deposition noticed in Untreated Cotton fabric.

### Drug Release Test Results

From the Table 2, it is clear that the release of herbal (natural agent extracts namely key components) from the fabric leaches in a uniform manner and also a good amount of drug is getting released from the fabric. In Figure 5, it is understandable that the drug release increases uniformly each day which shows that release of drug from the fabric is even and uniform and also it is proper.

### Antibacterial Test Results (Zone of Inhibition, Z.O.I)

The values given in the Table 3 denotes the zone of inhibition as a measure of Antibacterial activity of the Herbal Treated Cotton Dish Clothes. Z.O.I is the unit used to measure the antibacterial activity of the dish cloths. Figure 6 and 7 depicts the zone of inhibitions of the Cotton Fabric Samples finished with different proportions of Clove and Ginger against six different bacteria species identified in the Used Kitchen Dish Clothes. The results obtained clearly shows that the finished fabrics have good antibacterial activity and its inhibition zone varies from 4 mm to 6 mm against B1 bacteria, 3 mm to 7 mm against B2 bacteria, 2 mm to 4 mm against B3 bacteria, 2 mm to 3 mm against B4 bacteria, 1 mm to 2 mm against B5 bacteria and 1 mm to 4 mm against B6 bacteria.

### Statistical Analysis – One way ANOVA

The statistical analyses of Antimicrobial Test result are done by using SPSS software and the result was summarized in Table 4. From table, since the mean value and the F calculated value are greater in samples 3 and 5, it is proved that the samples finished with Ginger and Clove in the proportions of 50% and 50% respectively and Clove 100% has better antibacterial activity when compared to the other samples.

### Real Time Testing Results

In this testing, the natural extract treated cotton fabric after subject to usage in kitchens tested for the presence of bacterial contamination by using Agar diffusion method. Two samples finished with herbal extracts in the proportion of 25% Ginger and 75% Clove are provided to a vegetarian hotel kitchen and a non vegetarian hotel kitchen and asked them to use it for a period of ten days. Once tested in Agar test method, it is observed that there are no bacterial contaminations present in both the samples.

## CONCLUSION

The Cotton Terry Fabric commonly used as dish cloths in kitchens were provided to 6 places of vegetarian and non vegetarian kitchens that includes houses and hotels to use for a certain period of time. Six bacterial species were identified namely *Moraxella sp.*, *Staphylococcus sp.*, *Escherichia sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. The herbals *Syzygium aromaticum* (Ginger) and *Zingiber officinale* (Clove) after extracted by Soxhlet method were finished with Cotton Terry Fabric by pad-

dry-cure showed better antibacterial activity against the identified bacteria. The finished fabrics have good antibacterial activity and its zone of inhibition varies from 4 mm to 6 mm against B1 bacteria, 3 mm to 7 mm against B2 bacteria, 2 mm to 4 mm against B3 bacteria, 2 mm to 3 mm against B4 bacteria, 1 mm to 2 mm against B5 bacteria, 1 mm to 4 mm against B6 bacteria. The Ginger 50% and Clove 50% sample and Clove 100% samples have better antibacterial activity against all the bacteria when compared to the other samples. The evaluation of the herbal finish treated cotton dish cloths subjected to real time study in form of usage in kitchens for 10 days showed no bacterial growth by Agar diffusion method.

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