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Antimicrobial Activity Of Cotton Fabric Treated With Solanum Incanum Fruit And Red Onion Peel Extract.

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SCHOOL OF RESEARCH AND POSTGRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

ANTIMICROBIAL ACTIVITY OF COTTON FABRIC TREATED WITH SOLANUM INCANUM FRUIT AND RED ONION PEEL EXTRACT.

MASTER THESIS

BY

TESFA NEGA GESESE

Nov, 2019 **BAHIR DAR, ETHIOPIA**

ANTIMICROBIAL ACTIVITY OF COTTON FABRIC TREATED WITH SOLANUM INCANUM FRUIT AND RED ONION PEEL EXTRACT.

BY

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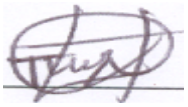
A thesis submitted to the school of research and graduate studies of Bahir Dar Institute of Technology, Bahir Dar University in partial fulfillment of the requirements for the degree of Masters of Science in process engineering under faculty of chemical and food engineering.

SUPERVISOR Dr.ir. SOLOMON W.

BAHIR DAR, ETHIOPIA, 2019 G.C

DECLARATION

I do hereby declare that this work has been originally carried out by me under the supervision of Dr.ir. Solomon.W., associate professor in chemical and postharvest technology, this work has not been submitted elsewhere for any other degree.

Student name: Tesfa Nega Gesese signature: 

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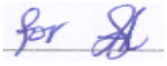
Name: Beteley Tekola (PhD) signature:  Date: 01/03/2012 E.C

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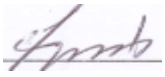
Name: Nigus Gabbiye Habtu (PhD) signature:  Date: 01/03/2012 E.C

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Dedicated to My Beloved Families

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ABSTRACT

The majority of the antimicrobial compounds used for treating textiles are synthetic based and are not considered to be environmentally friend. Therefore, solanum incanum fruit and onion peel were selected for the current study based on its potent antimicrobial activity. The active substance was extracted from fruit and peel by using the maceration extraction technique for 7 days with a mass liquor ratio of 1:10 (mass to solvent). The solanum incanum fruit and red onion peel extracts were applied, alone and together, on the cotton fabric samples by the pad-dry-cure method, using citric acid as a cross-linking agent. The antibacterial activity and the wash durability of the treated cotton fabrics were assessed by AATCC 100-2004 method. Among all treatments, the cotton fabric treated with 50:50 combination was found to be more in bacterial reduction. It was 100% and 99.92% bacterial reduction in cotton fabric with 5 g/l concentration for S. aureus and E. coli respectively. The wash durability of fabric treated with 50:50 combination was 85% for S. aureus and 84.17% was for E. coli bacteria after 15 wash cycle. After treatment, the tensile strength (ES ISO 13934), air permeability (ES ISO 9237), bending length (BS 3356), water absorbency (AATCC 79), and soil degradation were tested. Air permeability, water absorbency, and tensile strength were decreased. Soil degradation tests proved the biodegradability of the treated sample. The result recommended that the use of herbal extract can potentially be used as a substituent to a synthetic agent.

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ABBREVIATIONS

AATCC	American Association of Textile Chemists and Colorists
CFU	colony-forming unit
CLSI	clinical and laboratory standards institute
DW	dry weight
FTIR	Fourier transforms infrared spectroscopy
GAE	Gallic acid equivalent
MIC	minimum inhibitory concentration
MRSA	multidrug-resistant Staphylococcus aureus
PHMB	poly hex methylene biguanide
QUE	quercetin equivalent

1. INTRODUCTION

1.1. Background

The textile industry is a diverse, heterogeneous sector that involves several wet processes in the production of a wide variety of products. Despite their endless uses, they have some drawbacks mainly the susceptibility to microbial attack (Mohammad & Engineering, 2015). They provide several favorable conditions such as moisture, temperature, oxygen, and nutrients required for rapid growth and multiplication of pathogenic microorganisms (Mohammad & Engineering, 2015).

The growth of microorganisms on textiles had negative effects not only on the textile itself but also on the wearer (Gao & Cranston, 2008; Unango, Ramasamy, & Technology, 2019). Most of these negative effects are a reduction of mechanical strength in fabrics, discoloration of the fabric, likelihood contamination, generation of bad odor, allergic response and skin irritation (Gao & Cranston, 2008; Mohammad & Engineering, 2015; Purwar, 2019). Such a bottleneck problem forced attention towards developing advanced textile-based medical products. The rapid growth in medical and functional textiles provides many opportunities for the application of innovative functional finishes (Ali, Purwar, Joshi, & Rajendran, 2014). Antimicrobial finishes can be applied to textile substrates by the exhaust, pad-dry-cure, coating, spray method or spinning dope (Dhiman, Chakraborty, & Textiles, 2015; Purwar, 2019). The growth of microbes on the textile can be controlled by applying antimicrobial agents by various mechanisms like preventing cell production, blocking of enzyme reaction within the cell membrane to the destruction of cell wall and poisoning the cell from within (Dhiman et al., 2015; Doughari, 2012).

Mostly the textile sector utilized a synthetic antimicrobial agent against bacteria and fungi. But, most of a synthetic antimicrobial agent such as triclosan, formaldehyde are the potential to cause skin irritation, non-biodegradability and bioaccumulation effect (Tawiah, Badoe, Fu, & Europe, 2016). However, the herbal antimicrobial finishes overcome the disadvantages of the chemical finishes because they are eco-friendly, non-toxic and non-allergic (El-Shafei, Shaarawy, Motawe, & Refaei, 2017; Gopalakrishnan, Saravanan, & Europe, 2017). The antimicrobial effectiveness of the plant depends on the chemical structure of the active component present in the plant and their concentration (Nalankilli & Tadesse, 2018; Sathianarayanan, Bhat, Kokate, & Walunj, 2010; Thirumurugan, 2010). Today, numerous herbs and vegetables have been studied to extract the antimicrobial agent. Among the numerous natural herbs and vegetables, solanum incanum and onion are a source of antimicrobial agents.

Solanum incanum L. is one of the most important traditional medicinal plants which belong to the Solanaceae family (Abebe, Gebre, & Haile, 2014; Nalankilli & Tadesse, 2018). Solanum incanum is a plant characterized by thorny leaves, yellow fruits and blue flowers with yellow pistils which mostly distributed in the Horn of Africa as described by Nalankilli & Tadesse (2018).

According to (Sahle & Okbatinsae, 2017; Sambo, Pam, & Dahiru, 2000), the presence of photochemical constituent like alkaloids, saponins, flavonoids, terpenoids, steroids, cardiac glycosides, phenols and tannins present in solanum incanum plant made it be responsible for many pharmacological activities.

Onion (Allium cepa L) is a common food plant rich in several phytonutrients associated with the treatment and prevention of several diseases (Škerget, Majhenič, Bezjak, Knez, & quarterly, 2009). Allium cepa commonly called onion belongs to the family of Alliaceae and are grown in every part of the world where plants are farmed and exhibit great diversity in a form including color, shape, dry matter content and pungency (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). The variety also has an effect on the antimicrobial activity, since the secondary metabolite present in each variety (red variety, green variety and white variety) are different The bioactive compound from onion exhibited antibacterial and antifungal activities (Škerget et al., 2009).

1.2. Problem Statement

Majority of the synthetic antimicrobial agents utilized in the textile industry are leaching type; result in decreasing their concentration and fail to inhibit the growth of harmful microbes and the release of these agents' acts as poison to a wide spectrum of bacteria and fungi (Kumar, Raghu, Kumar, Varghese, & Kotresh, 2017). on the contrary, the textile industry looks for non-toxic, non-allergic and eco-friendly natural antimicrobial agents that do not adversely affect the quality of the textile material and the ecosystem as a substitute for synthetic toxic chemicals (Kumar et al., 2017). Nowadays, in Ethiopia cooks will almost always use red onion which results in discarded underutilized parts of the vegetable and it may cause an environmental problem like a source of unpleasant odor. But the onion peel has high phenolic and flavonoids content, which act as antimicrobial agents. On the other side, no study has been undertaken to investigate the combined antimicrobial effect of solanum incanum and onion peel extract on cotton fabric. Thus, to overcome these bottles' necked problem applying herb extract on the cotton fabric was taken as an immediate solution. For this study, the two herbs were applied by varying their proportion to examine the combined effects of the antimicrobial agent on the antibacterial activities, wash durability and physical properties of cotton fabric.

1.3. Objective

1.3.1. General objective

To investigate the antimicrobial activity of cotton fabric treated with solanum incanum fruit and red onion peel extract

1.3.2. Specific objective

- To determine the yield of methanolic extract of solanum incanum fruit and onion peel
- To identify the bioactive metabolites, present in the fruit of solanum incanum and in the peel of red onion with a chemical test

- To evaluate the physical, antibacterial, bio-degradability and laundering properties of the treated fabric

1.4. Scope of the Study

This research was focused on investigating effective and durable antimicrobial activity on cotton fabric for two different types of antimicrobial agents and with their standard combinations, i.e. red onion peel and solanum incanum fruit. Total phenol and total flavonoid content was determined using UV-Vis spectroscopy. The quantitative antibacterial assessment of the treated fabric was carried on against two challenged microorganism's staphylococcus aureus (S. aureus), a gram-positive bacterium, and Escherichia coli (E. coli), a gram-negative bacterium. The wash durability of the treated fabric also evaluated. The main idea of the study is analyzing the effect of antimicrobial agents on the physical properties of cotton fabric, wash durability and the potent antibacterial activity of the fruit of solanum incanum and the peel of red onion by AATCC 100-2004 standard method on cotton fabric.

1.5. Significance of the Study

Successful achievement of the research will able to play a great role by providing a remedy for the bottlenecked problems of environmental pollution and human being disease, by introducing low cost, easily available herb extracts antimicrobial agents. With this research, the synthetic antimicrobial agent was substituted by fruit and vegetable waste extract antimicrobial agents. In general, this research was signifying not only the textile sector but also the whole society.

2. LITERATURE REVIEW

2.1. Overview

The textile industry mainly concerned not only the design and the manufacturing of clothing but also the distribution and the use of textiles. The textile and clothing industry, normally seen as “traditional industry” is an important part of the European Asian manufacturing industry (Chandrasekar & Vijayakumar, 2017). Textiles material serve as medium to facilitate the growth of microorganisms such as bacteria and fungi (Kumar et al., 2017; Malpani, 2013; Murugesh Babu & Ravindra, 2015; Purwar, 2019; Şapcı, Yılmaz, Vural, Bahtiyari, & Benli, 2017; Singh, Punia, & Singh, 2017).

The growth of microorganisms on textiles generates basically two different types of problems depending on the type of fiber and environmental conditions. The first one is a problem with regards to a reduction of mechanical strength, discoloration, stains, which occur in fabrics made of natural fibers. The second type is associated with health and hygiene such as the generation of unpleasant odor, skin irritation, contamination for cross infections, etc. Such types of issues are generated in the fabrics of all types of fibers. For these reasons, it is necessary that the growth of microorganisms on textiles be inhibited during their application and storage(Kumar et al., 2017; Malpani, 2013; Singh et al., 2017).

Table 2.1: Representative class of microorganisms found on the fabric (Purwar, 2019)

S.No.	Species	Resulting Diseases or Condition	End Uses
Gram-positive bacteria			
1	Staphylococcus aureus	Pyogenic infections	Hygienic, medical
2	Staphylococcus epidermis	Body odor	Aesthetic
3	Corynebacterium diphtheroid	Body odor	Aesthetic
4	Bacillus subtilis		
5	Brevibacterium ammonia genes	Diaper rash	Hygiene
Gram-negative bacteria			
6	Escherichia coli	Infection of urogenital	Medical, aesthetic
7	Klebsiella pneumonia	Pneumonia	Medical
8	Proteus mirabilis	Urinary infections	Medical
9	Pseudomonas aeruginosa	Infection of wounds	Medical
10	Epidermophyton floccosum	Infection of skin	Hygiene
Fungus			
11	Candida Albicans	Diaper rash	Hygienic
12	Trichophyton interdigitates	Athlete's foot	Hygienic
13	Aspergillus Niger	Rotting	Degradation
14	Penicillium citrinum	Rotting	Degradation
15	Chaetomium globosum	Rotting	Degradation

2.2. Important Definition-Related Antimicrobial Textiles

Antimicrobial agent: It is an agent that either kills micro-organisms or inhibit their growth by interfering with the necessary mechanism of the microbe's cell (Purwar, 2019; Sood, 2014). These agents work either by the slow release of the active ingredient or by surface contact with the microbes (Sood, 2014).

Bacteriostatic agent: An agent that prevents the growth of bacteria, but does not necessarily kill them or their spores. Similarly, fungi-static describes an agent that stops the growth of fungi (Purwar, 2019).

Minimum inhibitory concentration: The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation referred to as minimum inhibitory concentrations (MICs) (Purwar, 2019).

Minimum bactericidal concentration: The lowest concentration of an antimicrobial that will prevent the growth of an organism after sub-culturing onto antibiotic-free media defined as minimum bactericidal concentrations (Purwar, 2019).

2.3. Microorganisms and Mode of Action of Antimicrobial Agents

A living microbe (e.g., bacterium, fungus) typically has an outermost cell wall which is mainly composed of polysaccharides as shown in Fig.2.1(Purwar, 2019). A. This cell wall maintains the integrity of cellular components and shields the cell from the extracellular environment. Immediately beneath the cell wall is a semi-permeable membrane that encloses intracellular organelles and a myriad of enzymes and nucleic acids.

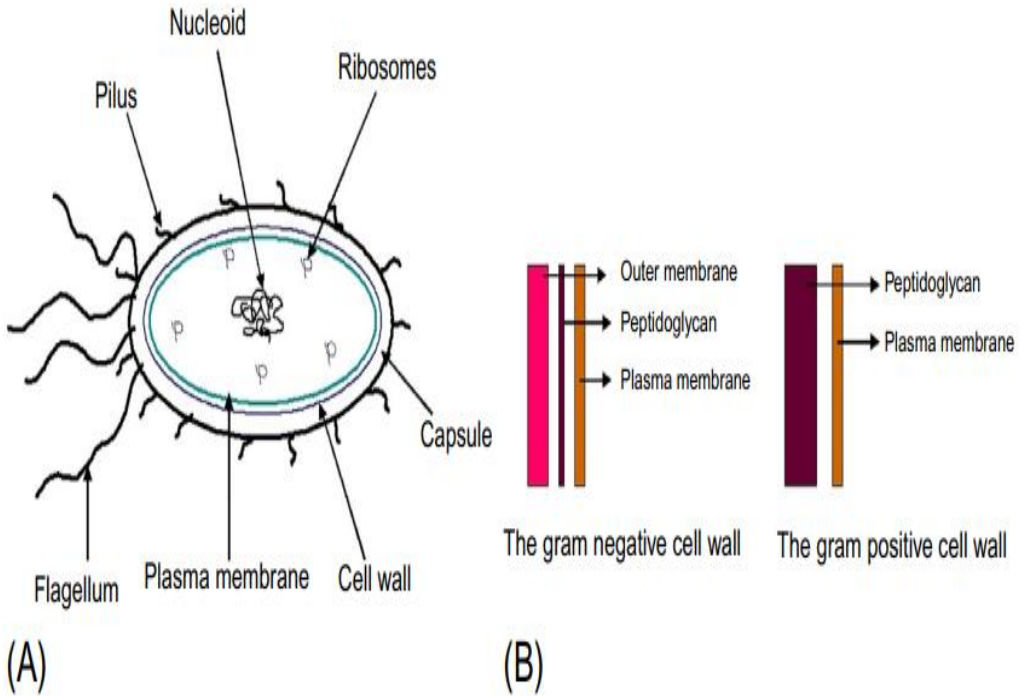


Figure 2.1: Schematic structure of (A) bacterial cell (B) difference in the cell wall of Gram-positive

The enzymes are responsible for the chemical reactions that take place within the cell, and the nucleic acids store all the genetic information of the organism. The survival or growth of microorganisms depends on the integrity of the cell and the concerted action and proper state of these components. The way the antimicrobial agent inhibits or kill can be attributed to damage the cell wall or alter cell membrane permeability, denature proteins, inhibit enzyme activity, or inhibit lipid synthesis. The bacteria can be divided into two major groups based on their response to the Gram stain procedure developed by Christian Gram. Gram-positive bacteria are stained purple, while the Gram-negative bacteria are colored pink or red by the gram staining technique. The gram-positive cell wall consists of a single 20–80 nm thick homogeneous peptidoglycan or murein layer lying outside the plasma membrane as shown in Fig.2. 1.B. The gram-negative wall is quite complex. It has a 1–3nm peptidoglycan layer surrounded by a 7–8 nm thick outer membrane. A periplasmic space is also found in gram-negative bacteria (Purwar, 2019).

2.4. Antimicrobial Agents Used for Textiles

The antimicrobial agent applied to the textiles basically classified into two groups based on their source of origin: (i) synthetic antimicrobial agent and (ii) natural antimicrobial agents. Metals and its salts, quaternary ammonium compounds, triclosan, poly hexamethylene biguanide (PHMB), regenerable n-halamine, and peroxy acids are the major synthetic antimicrobial agent applied on textiles and show excellent antimicrobial activity. The major concerns with synthetic antimicrobial agents are their strong biocidal activity towards no target microorganisms and the creation of water pollution (Purwar, 2019). Natural antimicrobial agents gained considerable attention in the field of medical and health care textiles due to properties such as being environment-friendly, skin-friendly, safe and non- toxic as compared to synthetic antimicrobial agents (Tadesse & Nalankilli, 2017).

The antimicrobial agents derived from natural sources are known as natural antimicrobial agent. Natural antimicrobial agents can further be classified into two major categories: (i) plant-derived antimicrobial agents and (ii) animal-derived antimicrobial agents (Purwar, 2019). Most of the reviewed paper in the current study focused on the natural antimicrobial agent derived from plant used for textiles applications (Ali et al., 2014; Cowan, 1999; Kasiri & Safapour, 2014; Kumar et al., 2017; Malpani, 2013; Mujeeb, Bajpai, & Pathak, 2014; Murugesh Babu & Ravindra, 2015; Şapcı et al., 2017; Simoncic & Tomsic, 2010; Singh et al., 2017; Tadesse & Nalankilli, 2017; Windler, Height, & Nowack, 2013).

2.5. Antimicrobial Compounds derived from Plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives (Chandrasekar & Vijayakumar, 2017). Most are secondary metabolites, of which at least 12,000 have been isolated, a number of estimated to be less than 10% of the total(Chandrasekar & Vijayakumar, 2017). In most cases, the active constituent present in the plant used as plant defense mechanisms against predation by microorganisms, insects, and herbivores. For instance, terpenoids, give plants their odors; quinone and tannins are responsible for

plant pigment. The terpenoids capsaicin from chili peppers is responsible for flavor and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

Phenolic compounds

Pyrocatechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms(Mohammed, 2006). Catechol has two –OH groups, and pyrogallol has three. The site(s) and the number of hydroxyl groups on the phenolic group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity(Mohammed, 2006). Caffeic acid had better antimicrobial activity than coumaric acid of the same structure due to the presence of one more hydroxyl group in caffeic acid on the phenolic rings. Thymol has better antibacterial activity than carvacrol due to the presence of –OH group in ortho position (Cowan, 1999; Doughari, 2012; Mohammed, 2006; Purwar, 2019). Phenolic compounds possess great structural variations and are one of the most diverse groups of secondary metabolites (Purwar, 2019). The chemical structure of the phenolic compound is presented below in Figure 2.2 (Purwar, 2019).

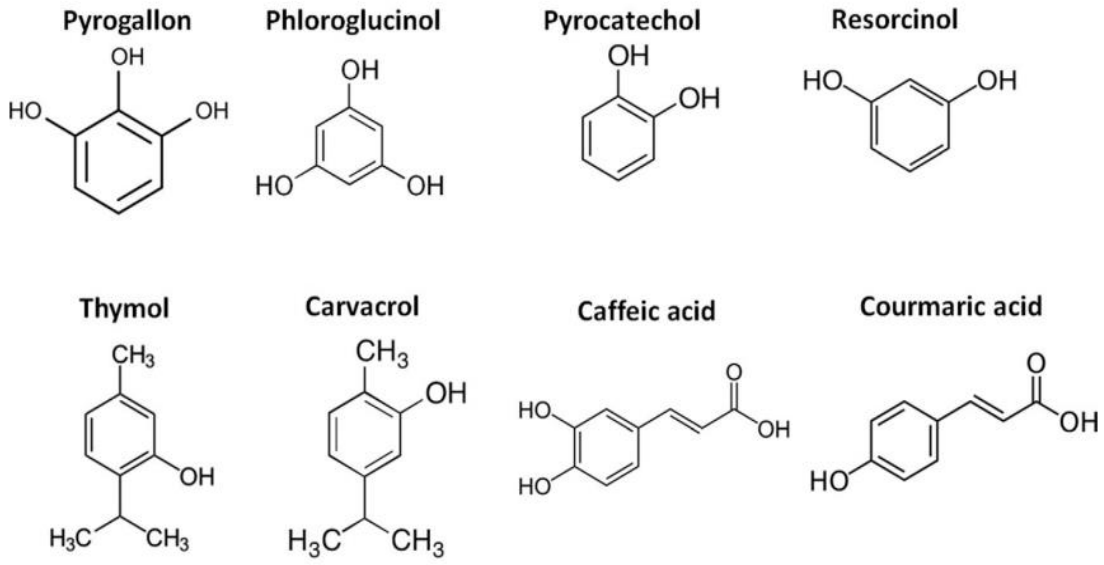


Figure 2.2: Chemical structures of some of the phenolic compounds sourced from Purwar, 2019.

Flavones, flavonoids, and flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl groups yields a flavonol(Cowan, 1999). Flavonoids also are hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Hence, they are known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to form a complex with extracellular and soluble proteins (Cowan, 1999; Doughari, 2012; Mohammed, 2006; Purwar, 2019). The more lipophilic flavonoids may also disrupt microbial membranes (Cowan, 1999; Mohammed, 2006; Purwar, 2019). Fig.2.3 shows some of the chemical structure of flavonoids responsible for antibacterial activity(Purwar, 2019).

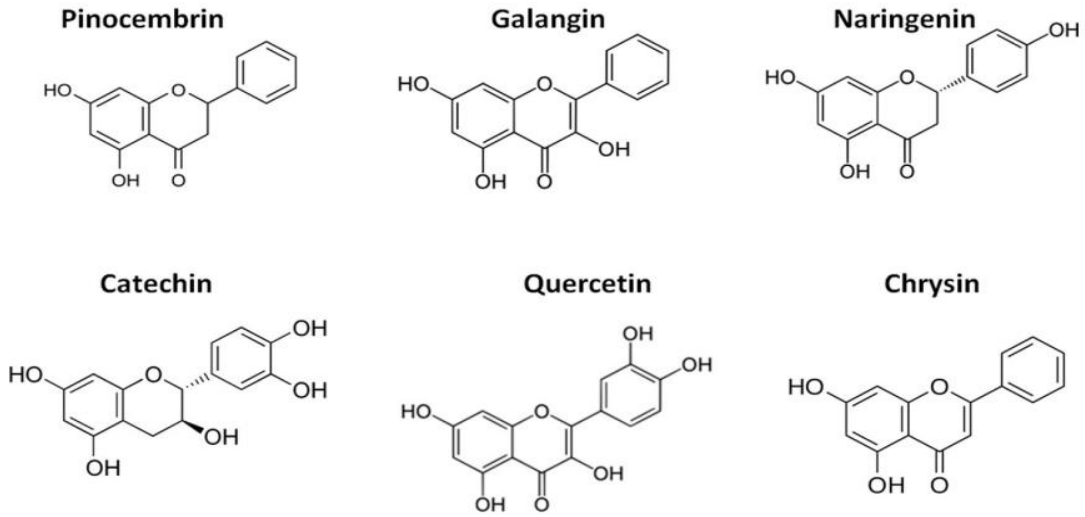


Figure 2.3: Chemical structures of flavonoids sourced from Purwar, 2019.

Quinones

Quinones are aromatic rings with two ketone substitutions as presented in Fig.2.4 (Purwar, 2019). They are ubiquitous in nature and are characteristically highly reactive. The switch between diphenol (and hydroquinone) and diketone (or Quinone) occurs easily through oxidation and reduction reactions (Purwar, 2019). In addition to providing a source of stable free radicals, quinones are known to form an irreversible

complex with nucleophilic amino acids in proteins, which leads to inactivation of the protein and loss of function(Cowan, 1999; Purwar, 2019).

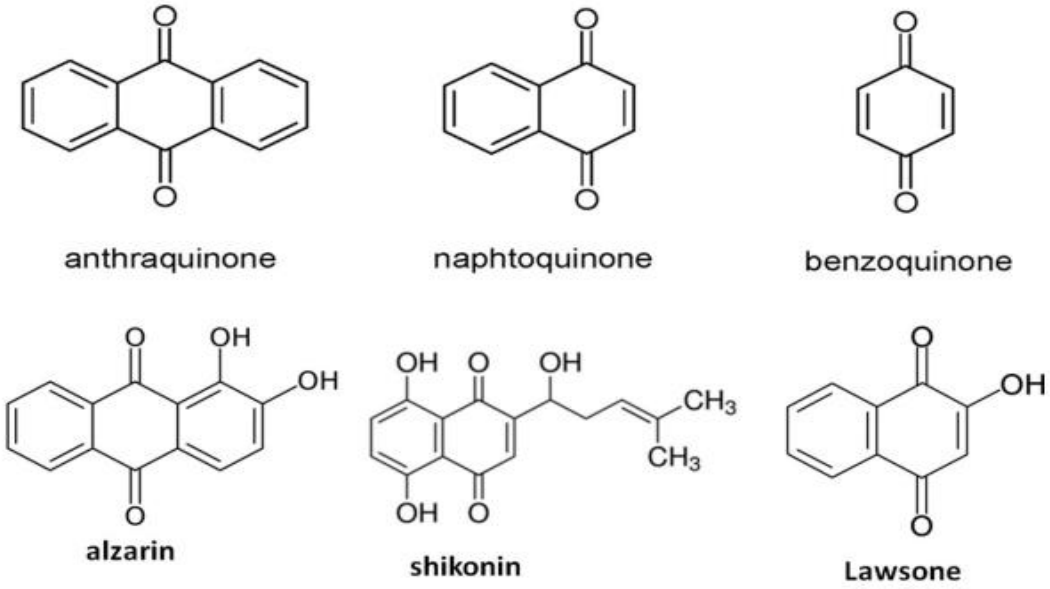


Figure 2.4: Chemical structures of quinines sourced from Purwar, 2019.

Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances(Mohammed, 2006). Their molecular weights range from 500 to 3000 and they are found in almost every plant part: bark, wood, leaves, fruits, and roots (Cowan, 1999; Doughari, 2012; Mohammad & Engineering, 2015; Mohammed, 2006; Purwar, 2019). Their antimicrobial activity is probably due to making irreversible complex with nucleophilic amino acids in proteins often leading to inactivation of the protein, enzyme, loss of function, inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Purwar, 2019). Tannins in plants inhibit insect growth and disrupt digestive events in ruminal animals (Cowan, 1999). The chemical structure of tannin presented in figure 2.5 (Purwar, 2019).

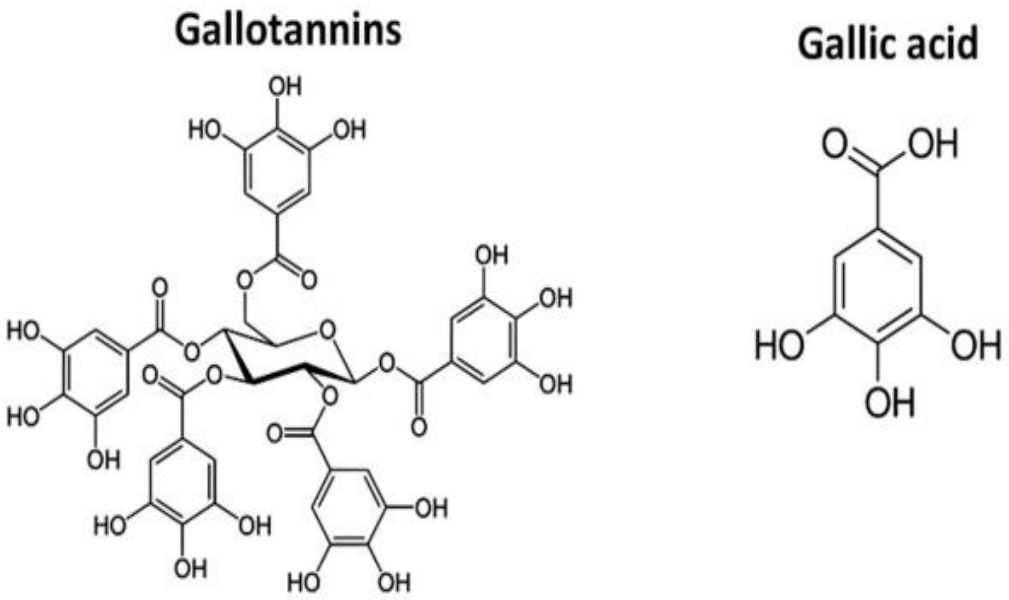


Figure 2.5: Chemical structures of tannins sourced from Purwar, 2019

Alkaloids

Alkaloids are heterocyclic nitrogen compounds. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy papaver somniferous; the name morphine comes from the Greek Morpheus, the god of dreams(Cowan, 1999; Mohammed, 2006).

Terpenoids

Terpenes or Terpenoids are active against bacteria, viruses, and protozoa. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Mohammed, 2006; Purwar, 2019).

Anthraquinones

Anthraquinones dyes from biological sources have been used for textile dyeing since antiquity. They are characterized by good fastness to light and have been extensively studied due to their great potential in the field of functional textiles (Mohammad &

Engineering, 2015). Some of the chemical structure of anthraquinone are presented in figure 2.6 (Purwar, 2019).

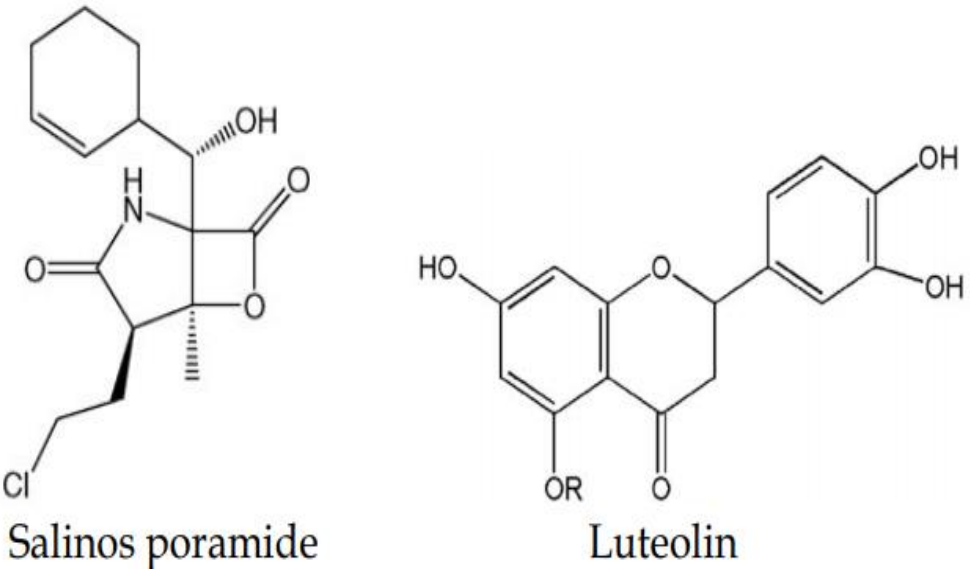


Figure 2.6: Basic structures of some pharmacologically important plant-derived anthraquinones sourced from Purwar, 2019

Saponins

The term Saponins is derived from Saponaria vaccaria (quillaja Saponaria), a plant, which abounds in saponins and was once used as soap. Saponins, therefore, possess 'soap like' behavior in water, i.e. they produce foam. Saponins are regarded as high molecular weight compounds in which, the sugar molecule is combined with triterpene or steroid aglycone. There are two major groups of saponins and these include steroid saponins and triterpene saponins.

Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for the activity of cardiac glycosides (Doughari, 2012).

Glycosides

Glycosides in general, are defined as the condensation products of sugars (including

polysaccharides) with a host of different varieties of organic hydroxyl (occasionally thiol) compounds (invariable monohydrate in character), in such a manner, that the hemiacetal the entity of the carbohydrate must essentially take part in the condensation. Glycosides are classified on the basis of the type of sugar component, chemical nature of aglycone or pharmacological (Doughari, 2012).

2.6. Antibacterial Properties of Plants

Thirumurugan (2010) investigated on phytochemical and antibacterial properties of methanolic extract of six Indian folk medicinal plants. The finding showed that out of six plants tested for antimicrobial activity, five plant species showed antibacterial activity by inhibiting one or more microorganisms.

Alkhalifah & Science (2016) provided antimicrobial detail of hot water and organic solvent extract of three plants, used traditionally pathogenic microbes. The finding proved that the three methanolic extract plants had the greatest antimicrobial activity against both Gram-positive and Gram-negative bacteria.

Indhumathi & Mohandass (2014) studied the efficacy of ethanolic extract of solanum incanum fruit extract for its antimicrobial activity. The findings showed the rich availability of alkaloids, tannin, flavonoids, phenols, glycosides, carbohydrate, steroid, triterpenoids and resin as secondary metabolites in the solanum incanum fruit. The zone of inhibition produced by the crude ethanol extract was ranged from 10-26 mm, at a concentration of 100 µg/disc. The crude ethanol extract showed the highest antibacterial activity (26 mm) against Staphylococcus aureus and also showed good antibacterial activity (10 -25 mm) against all pathogenic bacteria.

Mujeeb et al. (2014) focused on phytochemical evaluation, antimicrobial activity, and bioactive component determination by studying the aqueous and methanolic leaf extract of 18 varieties/ accessions of aegle marmelos. The crude extract of A. marmelos revealed the presence of several biologically active phytochemicals with the highest quantity of alkaloids, flavonoids, and phenols in pant aparna variety. The antibacterial efficacy was investigated against pathogenic bacteria strain. The aqueous extract

showed the highest inhibitory activity against S. epidermidis, whereas methanolic extract was found to be potent against S. aureus at 40 mg/ml concentration. However, in aqueous: ethanol, the best results were observed against E. aerogenes followed by K. Pneumonia and S. epidermidis. The MIC of aqueous and methanol extract of Aegle marmelos ranged from 10 to 40 mg/ml, whereas aqueous: ethanol ranged between 40 and 160 mg/ml. The GC-MS analysis revealed the presence of many bioactive compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolic and steroids which can be postulated for antibacterial activity.

Jepkoech & Gakunga (2016) studied on the antimicrobial activity and phytochemical screening of methanolic extract of solanum incanum fruit against clinical samples of Staphylococcus aureus. The phytochemical screening result revealed the presence of alkaloids followed by saponins and glycoside strongly. The antimicrobial test showed zones of inhibition produced by the different concentrations of the methanolic extract against S. aureus ranged from 6.0 mm to 12.5 mm and for the aqueous extract which had much smaller zones of inhibition ranged from 6.0mm to 9.0mm.

Sahle & Okbatinsae (2017) investigated the phytochemical and antimicrobial activity of the fruit extract of solanum incanum grown in Eritrea. The fruit extract was identified to have alkaloids, flavonoids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins. The antibacterial activity of the plant extract was assayed in vitro by agar well diffusion method against the three bacterial species (E. coli, S. aureus and S. typhimerium) and a fungus (C. albican). The maximum antimicrobial activity was shown by E. coli, S. typhimerium followed by C. albicana respectively, and a minimum activity was shown by S. aureus.

Ortiz & Technology (2015) carried out the antimicrobial activity of onion and ginger against two foodborne pathogens Escherichia coli and staphylococcus aureus. The finding revealed the raw onion extract showed greater antimicrobial activity against S. aureus with an MBC concentration of 70% v/v and an inhibition zone of 28mm been the largest.

2.7. Herbs Used in the Present Study

Based on their availability in the country and the highest antimicrobial resistance reviewed from literature solanum incanum fruit and red onion peel were selected in the case of the present study.

2.7.1. Solanum incanum

Solanum incanum (bitter or sodom apple) belongs to the solanaceae family (Doughari, 2012; Sahle & Okbatinsae, 2017; Sambo et al., 2000). It is one of about 1,500 Solanum species in the world. Widely distributed in the Horn of Africa and it shows the characteristic thorny leaves, yellow fruits and blue flowers with yellow pistils (Abebe et al., 2014; Tadesse & Nalankilli, 2017; Waweru, Muthamia, & Otaye, 2017). Many scholars have been reported that solanum incanum used traditionally to treat pathogenic microbes (Abebe et al., 2014; Alkhalifah & Science, 2016; Nalankilli & Tadesse, 2018; Waweru et al., 2017). In fact, utilizing herb extract for the antimicrobial agent is more advantage over synthetic antimicrobial agents (Ashokkumar, Ramaswamy, & Sciences, 2014).

Abebe et al. (2014) and Nalankilli &Tadesse (2017) explained that throughout tropical Africa a sore throat, angina, stomach- pain, colic, headache, painful menstruation, and liver pain is treated with Solanum incanum. For this purpose, leave, fruit and root decoctions are drinking, roots are chewed and sap swallowed, leaf sap is used for washing painful areas, and ash of burnt plants is mixed with fat and applied externally. They also explained that the fruit of solanum incanum is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania. In Niger, Sudan, Rwanda, and Namibia the fruits are used as an ingredient of arrow poison and in Mozambique of fish poison. In Ethiopia, the fruit juice is used by peasant farmers to control ticks. The boiled fruits are used as soap and in tanning leather.

To sum up, Nalankilli & Tadesse (2018) have been reported that a high antimicrobial effect on cotton fabric achieved with a concentration of 30g/l Solanum Incanum fruit

extract by disk diffusion method. The zone of inhibition that was measured with 30g/l concentration for S. aureus was80mm or 8cmand for E. coli was 70mm or 7cm.

2.7.2. Onion

Onion (Allium cepa L.) is botanically included in the Liliaceous and species are found across a wide range of latitudes and altitudes in Europe, Asia, N. America, and Africa. Locally it called red onion (in Amharic key shinkurt). Onions are grown in every part of the world where plants are farmed and exhibit great diversity in the form including color, shape, dry matter content and pungency (Griffiths et al., 2002).

Škerget et al. (2009) investigated antioxidant, radical scavenging and antimicrobial activities of red onion (Allium cepa L) skin and edible part extracts. The result revealed that skin extracts showed significantly stronger inhibitory effects against B. cereus, E. coli, and P. fluorescens tested bacteria strains than the edible part extracts, and the inhibition activity increased with increasing concentration of extracts. This can be explained by the higher concentration of active compounds in skin extracts.

Ramos et al. (2006) studied the antibacterial activity of onion and the results of the screening of antibacterial activity using the disk diffusion test two MRSA strains and two H. pylori strains; and broth dilution test against gram-positive, gram-negative and C. albicans; the onion extract was relatively sensitive to S. aureus, whereas very resistance to E. coli and C. albicans.

(Lines & Ono, 2006; Silva et al., 2018) has been reported that, a beverage containing flavonoids extracted from onion peel, showed unexpected improvement of male sexual function. Singh et al. (2017) stated that extraction from different plant parts might be used in combination to achieve improved antimicrobial potency.

Ifesan (2017) studied the chemical composition of onion peel (Allium cepa) and its ability to serve as a preservative in cooked beef. The result revealed that onion peel extract could act as a bacteriostatic agent in meat. Bacillus cereus was the most sensitive to the extract, followed by E. coli, S. aureus, Proteus vulgaris, and B. subtilis.

(Eltaweel, 2013) done on assessment of antimicrobial activity of onion extract (Allium cepa) on Staphylococcus aureus; in vitro study. The result revealed that the methanolic suspension of Allium cepa at 1000 µg/ml was found to be more effective than the other concentrations with an inhibition zone reached to 29 mm.

In general, Singh et al. (2017) have been reported that applying onion peel extract on cotton fabric by exhaust method with 3g/l and 5g/l concentrations, shows 97.00% and 97.58% reduction respectively. On the other side, applying 50:50 combinations of pomegranate and onion peel extract exhibited maximum bacterial reduction i.e. 98.58% with 3g/l and 98.75% with 5g/l concentration by exhaust method.

In this study a lot of paper was reviewed, but, most of the reviewed papers concentrate on the technical details of applying individual natural agents, such as neem extract, chitosan, tea tree and aloe Vera on the textile finish. Furthermore, no study has been undertaken to investigate the combined antimicrobial effect of solanum incanum and onion peel extract on cotton fabric. Thus, the primary aim of the present study was to investigate the combined antibacterial effect of solanum incanum fruit and red onion peel extract on cotton fabric.

2.8. Extraction Techniques for Plant Extract

Extraction defined as a separation of medicinally active portions of a plant using selective solvents through standard procedures (Azwanida, 2015).

Azwanida (2015), Mohammed (2006) and Purwar (2019) explained different methods of extraction used to prepare extracts from medicinal plants. The natural antimicrobial extract mainly extracted from various parts of the plants such as bark, leaf, root, fruit, seed, and flower (Cowan, 1999; Kasiri & Safapour, 2014; Purwar, 2019). There are different methods to extract these materials from the natural sources described as follow:

Maceration: It involved soaking of grinding powder with a solvent and allowed to stand at room temperature for a period of a minimum of 3 days with frequent agitation

(Azwanida, 2015) followed by filtration and solvent evaporation (Sood, 2014). Repeated maceration may be more efficient than a single maceration. Since an appreciable amount of active plant extracts contain several ingredients along with active components principles may be left behind in the soaked plant material after pressing for the first time during the maceration process (Sood, 2014).

Soxhlet extraction or hot continuous extraction: In this method, a finely ground sample is placed in a "thimble" made from a strong filter paper or cellulose, which is a place, is in the thimble chamber of the Soxhlet apparatus. Extraction solvents are heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued (Azwanida, 2015). The process is repeated until the powder decolorizes (Sood, 2014).

Ultrasound-assisted extraction (UAE): It involves suspending the pulverized material in the solvent and subjecting it to cavitation's' under the influence of ultrasonic vibrations (Sood, 2014). The physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall; facilitating the release of compounds and enhancing mass transport of the solvents into the plant cells (Azwanida, 2015). After the filtration and evaporation of the water, the dry extract can be obtained (Sood, 2014).

Microwave-assisted extraction (MAE): Microwaves are made up of two oscillating perpendicular field's i.e. electric field and magnetic field and the former is responsible for heating (Sood, 2014). In microwave-assisted extraction, the microwave radiation interacts with dipoles of polar and polarizable materials (solvents and sample) causes heating near the surface of the materials and heat is transferred by conduction. The dipole rotation of the molecules induced by microwave electromagnetic disrupts hydrogen bonding which enhancing the migration of dissolved ions and promotes solvent penetration into the matrix. In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only. MAE can be considered as selective methods that favor polar molecules and solvents with a high dielectric constant (Azwanida, 2015).

2.9. Application and Assessment of Antibacterial Activity on Fabrics

Textile testing will definitely help in evaluating the effectiveness of antibacterial finishes and also can be used to conclude the quality of end products and their extent of microbial resistance(Palmer, 2016).

Singh et al. (2017) studied bacterial resistance to finish on cotton fabric with pomegranate and onion peel extracts by applying alone and with their standard combination by exhaustion method. The antibacterial efficacy of the treated fabric was performed against Gram-negative bacteria (E. coli) by the AATCC 100-2004 test method. It was observed that the antibacterial activity the fabric finished with 50:50 combinations of onion and pomegranate peel was better than the fabric finished with pure extract and any other standard combination of onion and pomegranate peel.

Sood (2014) investigated on application of herbal extracts on covering the fabric of sanitary napkins for bacterial resistance by a pad-dry-cure method. All the treated fabrics were assessed for their bacterial resistance property using standard quantitative test method AATCC Test Method 100. The bacterial resistance of the control and treated samples were tested against Gram-positive bacteria Staphylococcus aureus (Isolate1) and Gram-negative bacteria Pseudomonas spp. as per the test method. The result revealed that the zone of inhibition observed with methanolic extract of Bauhinia veriagata was 7 mm for Staphylococcus aureus and 8 mm for Pseudomonas spp., while its aqueous extract did not show any activity against the test bacteria. It was observed that the bacterial resistance subsequently increased with an increase in the concentration of the herbal extract derived from Woodfordia fructicosa.

Chandrasekar & Vijayakumar (2017) studied the application of chitosan-herbal composites for the fabrication of antimicrobial cotton fabric used in health care textiles by exhaustion, dip dry and pad-dry-cure method. The antibacterial activities of the treated and untreated fabrics were assessed against E. coli and S. aureus as recommended by AATCC. The finding showed that the antibacterial activity was highest in the fabrics finished by the pad-dry cure method. The maximum zone of inhibition was obtained by fabrics coated with herbal chitosan nanocomposites by pad-

dry cure method (26 mm), followed by fabrics coated with chitosan nanoparticles by pad-dry cure method (14 mm). The fabrics finished by other finishing methodologies also showed an antibacterial effect but comparatively lower. The least antibacterial effect was observed in the fabrics finished with the dip dry method in the case of the agar diffusion method. In the case of the percentage reduction test method, maximum antibacterial activity with the highest bacterial reduction percentage was observed for the fabrics coated by the pad-dry cure method. The bacterial reduction percentage for the fabrics finished by exhaustion method was found to be comparatively lower (herbal chitosan nanocomposites finished fabric -73%, chitosan nanoparticle finished fabric-46%) and the lowest percentage of bacterial reduction was recorded for the fabrics finished by dip dry method (herbal chitosan nanocomposites finished fabric -65%, chitosan nanoparticle finished fabric-39%.

Palmer (2016) stated shat AATCC 100 is a quantitative test method that gives a numerical estimation of the bactericidal and bacteriostatic activity of a fabric. Although this is helpful in comparing levels of antimicrobial activity among fabrics, it can be difficult if the fabric is hydrophobic (in order to efficiently inoculate) and does require the researcher to decide on "success criteria" which does not allow for inter-institution comparisons.

Purwar (2019) explained that some of the natural antimicrobial agents have very little affinity towards textile fibers. Because of this, such agents are applied through the textile finishing process, i.e., pad-dry-cure method along with the cross-linking agent. He also stated that the concentration of the antimicrobial agent varies in the range of 1–15% (w/v) pad- dry-cure method is effective. Since the antimicrobial activity of finished fabric depends on the bonding of a cross-linking agent with cellulosic fiber.

Gopalakrishnan et al. (2017) investigated the antimicrobial activity of coleus ambonicus herbal finish on cotton fabric using the direct exhaust method, microencapsulation method, and nano-encapsulation method. The bacterial reduction percentage of the finished fabric was assessed quantitatively by the AATCC agar diffusion test method 100. The result revealed that the samples finished by the direct exhaust method show a better degree of bacterial reduction percentage for both gram-

positive (Staphylococcus aureus) and gram-negative (Escherichia coli) than the microencapsulation and nano-encapsulation methods before washing. The samples finished by the direct exhaust, microencapsulation and nano-encapsulation methods possess a higher bacterial reduction percentage against gram-positive microbes than gram-negative. This indicates that coleus ambonicus herbs are very active against gram-positive rather than against gram-negative.

2.10. Assessment of Finish on Physical Properties and Wash Durability of Finished Fabrics

Sood (2014) studied the effect of herbal antibacterial treatments on the physical and performance properties of the treated non-woven fabrics. The results revealed that weight, bending length, the thickness of the treated fabric increase as compared to the untreated fabric. The air permeability and wettability of the treated samples decrease after the application of the herbal extract compared to untreated controls. It was observed that with the increase in fabric weight, the air permeability decreased.

Chandrasekar & Vijayakumar (2017) conducted a study on the application of chitosan-herbal composites for the fabrication of antimicrobial cotton fabric used in health care textiles. The durability of finish to washing was analyzed by washing all finished samples in the 'Launder-o-meter' by using standard ISO: 687-1979 (test no.1). The washing was done up to 40 laundering cycles and results were evaluated by after counting the bacterial colonies upon incubation. It was observed that herbal chitosan nanocomposites treated fabrics sustained their maximum antibacterial activity against both the test bacteria until 20 industrial washes. After that, there occurred a slight reduction in the activity of the fabric coated with the herbal chitosan nanocomposites due to the uniform coating, better affinity and sustained release of the herbal chitosan nanocomposites. Bulk finished fabrics showed relatively low air-permeability than the herbal chitosan nanocomposites finished fabrics. The tensile strength of herbal chitosan nanocomposites finished cotton fabrics were found to be similar to untreated control cotton fabrics. Whereas, the tensile strength of the bulk treated fabric showed a slight increment as compared to the untreated control.

Ali et al. (2014) evaluated the antibacterial properties of aloe Vera gel-finished cotton fabric. The durability of the Aloe Vera finished fabric was evaluated after the repeated washing cycle. It was found that the antibacterial activity retains more than 70% up to 5 machine washes and more than 50% even after 8 machine washes although there is a sharp reduction in antibacterial activity. The tensile strength of the finished samples decreased compared to the untreated sample. The bending length (stiffness) and crease recovery of the treated sample increased after the application of the extract as compared to the control(untreated).

Gopalakrishnan et al. (2017) investigated the antimicrobial activity of coleus ambonicus herbal finish on cotton fabric using the direct exhaust method, microencapsulation method, and nano-encapsulation method. The durability of the finishing was assessed by analyzing the antimicrobial activity of the washed sample. The finding showed that a marked reduction in the bacterial reduction percentage was observed in the sample finished by the direct exhaust method when increasing the wash cycles. The bacterial reduction percentage was 15 and 12 for S. aureus and E. coli, respectively, after 10 wash cycles, confirming that the direct method of application has poor wash durability of finishing. The micro and nano encapsulated samples show better resistivity in both gram-positive and gram-negative microbes than for the direct method after washing. It is evident that the nano encapsulated and microencapsulated samples have restricted the release of anti-microbial agents.

However, the nano encapsulated method displays good antimicrobial activity in terms of bacterial reduction percentage up to 20 wash cycles and shows moderate antimicrobial activity after 30 wash cycles, with both gram-positive and gram-negative microbes. It reveals that the nano encapsulated sample exhibits good wash durability in comparison to the microencapsulation and direct exhaust methods.

3. METHODS AND MATERIALS

3.1. Materials and Chemicals

Blood and MacConkey agar were used as a media of growing bacteria. The citric acid (99.5%) was used as a cross-linking agent. Standard soap 5g/l was used for washing to test antimicrobial effect after washing. Chloroform (99.8), hydrochloric acid (37%), ferric chloride (99%), ammonia (35%), aluminum chloride (99%), sulfuric acid (98%), acetic acid glacial (99.5%), potassium iodide (98.5%), phosphoric acid (85%)and sodium hydroxide (98.8) were used for phytochemical analysis. Gallic acid (99%), Folin- Ciocalteau reagent and sodium carbonate (99.5%) were used for total phenol content determination. Methanol (99.8%) was used for extracting the active component from the peel and fruit of solanum incanum and red onion. Quercetin acid (99.8%), sodium nitrile (99.5%), aluminum chloride, sodium hydroxide was used for total flavonoid content determination. All chemicals used in the current study were analytical grades.

3.2. Equipment used

Conical flask was used for preparation of different media, test tube for solution preparation of phenol and flavonoid analysis, padding mangle (Mathis made in Switzerland) for imparting antimicrobial agent onto cotton fabric, oven dryer for removing excess water from fabric after applying finishing agent, multifunctional grinder (RRH-100g) for reducing the size of the solanum incanum fruit and onion peel into powder form, universal strength tester (MESDAN, Italy) for measuring the tensile strength and elongation of break of the treated fabric, Rotary evaporator (Rota-vapor, RE300, UK) for concentrated the filtrate of the extract, oven (Bernareggio, M40-VF, Italy) for drying of sample, electronics balance for weighing sample, orbital shaker (Heidolph,Unimax2010, Germany) for extraction .

3.3. Experiment

3.3.1. Sample selection

Table 3.1: List of plant/vegetable selected for the present study

S. No	Plants /vegetable	Scientific name	Local name	English name	Family	Part used
1		Solanum incanum	Imbway	Soda apple	Solanaceae	Fruit
2		Allium cepa L.	Key shinkurt	Onion	Alliaceae	Peel

An exhaustive list of plants and vegetables explained in appendix 3.1 having antibacterial properties was prepared on a review basis. From the prepared list, one herbal plant parts i.e. solanum incanum fruit and one vegetable i.e. onion peel were selected as shown in Table 3.1, because of easy accessibility and their antimicrobial resistance due to the presence of mainly phenolic and flavonoid compound.

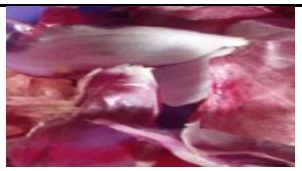
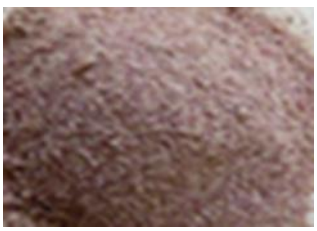
3.3.2. Sample collection and pretreatment

The plant material, solanum incanum fruit was collected from Abay Mado, kebele 11, around Giyon secondary and preparatory school, Bahir Dar, Amhara regional state, Ethiopia. The red onion peel was collected from Bahir Dar institute of technology student cafeteria. The collected sample from the available area was washed in tap water and rinsed in distilled water to remove dust and other impurities. Then the rinsed samples were dried in shade; solanum incanum fruit for five days and onion peel for 14

days by cutting it into the smaller piece using knife until its moisture content reduced to 14 %.

The dried samples were subjected for size reduction to powder (finely) by high-speed multifunctional grinder (RRH-100g, Hongtaiyang Electrical & Mechanical Service Co. Ltd of Yongkang City of Zhejiang Province) purchased from the local market, Bahir Dar, Ethiopia. After that, the powder was sieved with a sieve size of 0.5 mm to remove the oversized particles. Finally, the perspective powdered samples were stored in an airtight glass container at room temperature until used.

Table 3.2: Fresh, dried parts and dry powder of the selected plant/ vegetable

Plants/vegetable Conditions	Solanum incanum (fruit)	Red onion (peel/ skin)
Fresh		
Dried		
Dry powder		

3.3.3. Extract preparation

Extraction was done using a method described by (Harborne, 1998). The dried powder, 50 grams of each sample was soaked in 500 ml of absolute methanol with a continuous

shaking with an orbital shaker at 172 rpm for one week at room temperature. After a week, the perspective sample was filtered through What man filter paper (125 mm, No.1) using suction filter apparatus, while the residues were allowed for a second extraction. After filtration, the filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature of 50℃. The crude extracts were collected and dried in an oven at a temperature of 35℃ for four weeks. The dried concentrate was weighted, percentage yield determined and scooped into a well-labeled plastic bottle and stored in a refrigerator at 4℃ waiting for bioassay.

3.3.4. Percentage yield determination of the extracts

The percentage yield of solanum incanum fruit and red onion peel extract was determined using the method described by (Jepkoech & Gakunga, 2016).

$$\text{Percentage yield} = \frac{W1}{W2} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.1$$

Where w_1 is the dry mass of the extract, whereas w_2 is the dry mass of the sample before extraction.

3.3.5. Phytochemical analysis/screening

Chemical test: The chemical analysis of both the extracts was performed by following the protocol of (Indhumathi & Mohandass, 2014; Jepkoech & Gakunga, 2016; Kokate, Purohit, & Gokhale, 1996; Sahle & Okbatinsae, 2017; Sambo et al., 2000).

Test for Tannins: Ferric chloride test: 200 milligrams of extract from each sample was boiled in 10 ml of distilled water and filtered. A few drops of 5% ferric chloride solution were added to the filtrate. The formation of a blue-black precipitate confirms the presence of tannins.

Test for Flavonoids: Alkaline reagent test: From each sample, one milliliter of the extract was mixed with one milliliter of ten percent sodium hydroxide solution. The

formation of an intense yellow color, which becomes colorless on the addition of 2 ml of dilute acid (1 N HCl), indicated the presence of flavonoids.

Ammonium test: 2 ml of extract from each sample was shaken with 1 ml of 1dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was not observed at the ammonia layer which indicates the absence of the flavonoid from the plant extracts.

Aluminum chloride test: 2 ml of extract from each sample were shaken with 1 ml of 1 % aluminum chloride solution and observed for the light yellow color, which did not appear indicating the absence of flavonoids. The light-yellow color indicates the presence of flavonoid and when diluted with NaOH and HCl the yellow solution turns colorless.

Test for Anthraquinones: About half a gram of the extract from each sample was placed in a dry test tube and five ml of chloroform was added and shaken for five minutes. Then, the content was filtered and an equal volume of 100% ammonia solution was added and mixed together. A pink violet or red color in the ammonia layer indicating the presence of anthraquinones, which did not appear indicating the absence of anthraquinones.

Test for Saponins: Frothing test: To 10 ml of distilled water, 1 ml of filtrate from each extract was added and shaken vigorously for 10 minutes in a gradual cylinder. The formations of stable foam confirm the presence of saponins.

Test for Steroids: Salkowski test. 200mg of the extract powder from each sample was dissolved in 2ml of chloroform. A few drops of the concentrated sulfuric acid were added to the chloroform solution of the extract carefully and shaken well and allow to stand for a few minutes. On standing the formation of the red color indicating the presence of steroids.

Test for Alkaloids: **Wagner's reagent test**: 1 ml of each extract was warmed with 5 ml of 2% of sulfuric acid on a water bath for two minute and a few drops of Wagner's

reagent were added. The formations of reddish-brown colored precipitate confirm the presence of the alkaloids.

Test for Glycosides: **Sodium hydroxide reagent**: 2 ml of alcoholic extract from each sample was mixed with 5ml of water and a few drops of sodium hydroxide were added. The appearance of a yellow color confirms as an indication for the presence of glycosides.

Test for Terpenoids: Salkowski test. Two milliliters of chloroform and three milliliters of concentrated sulfuric acid were added to 1ml extract from each sample carefully. A reddish-brown coloration signifies the presence of Terpenoids.

Test for Phenols; Ferric chloride test: To 1ml of solanum incanum fruit and onion peel extract, 2.0ml of distilled water followed by drops of 1% aqueous FeC13 solution were added. The formation of bluish-black indicates to confirm the presence of phenols.

Test for resins: To 10 ml of acetic acid, 2 ml of methanolic extract of solanum incanum fruit and onion peel followed by a drop of concentrated sulfuric acid was added. The formation of purple color, which rapidly changed to violet, confirms the presence of resins.

Test for proteins: Xanthoproteic test: 2 ml of extracts from each sample were treated with a few drops of concentrated nitric acid. The formation of a yellow color precipitate indicates the presence of proteins.

Test for Quinone: The hydrochloric acid test: 2 ml of extracts from each sample were treated with concentrated hydrochloric acid and observed for the formations of yellow color precipitate confirm the presence of quinone.

FT-IR Spectroscopic Analysis: Fourier transform infrared spectrophotometer (FTIR) is the most powerful tool for identifying the type of chemical bonds/ functional groups present in the plant extract. The chemical bonds in the annotated spectrum characterized by the wavelength of light absorbed. Dried powder of methanolic extract of solanum

incanum fruit and red onion peel was used for FT-IR analysis. 10 mg of each dried extract powder was encapsulated in 100 mg KBr pellet, in order to prepare the translucent sample disks. The powdered sample of each extract was loaded in FT-IR spectroscopy, with a scan range from 4000 to 400 cm-1 with a resolution of 4 cm-1.

Quantification of total phenol content: The quantity of total phenolic contents in each extract was determined accordingly to the Folin-Ciocalteau reagent (FCR) method described by (Singleton, Rossi, & Viticulture, 1965) using Gallic acid as standard**.** Reagents: Folin-Ciocalteu's phenol reagent was prepared under the hood following the procedure described below: 10 g sodium tungstate ($Na_2WO_4.2H_2O$**)** and 2.5 g sodium molybdate ($Na_2M_OO_4.2H_2O$) were dissolved in 70 mL water. 5 mL of 85% phosphoric acid (H_3PO_4) and 10 mL concentrated hydrochloric acid (HCl) was added to the solution and boiled for 10 h. Then the following reagent was also added: 15 g lithium sulfate ($LiSO_4.H_2O$), 5 mL water and 1 drop bromine (Br_2). Refluxed the solution for 15 min to remove the excess bromine. The bromine oxidizes any trace of molybdenum-tungsten blue, and the final reagent should be yellow without any green. The refluxed solution was cooled to room temperature and brought to 100 ml with water. Briefly, in a 20 ml test tube, 1000 µL of the extract was mixed with 5000 µL of 1:10 diluted Folin–Ciocalteau reagent. The solutions were mixed thoroughly and incubated at room temperature (25ºC) for 5 min. After incubation, 4000 µL of 7.5% sodium carbonate (Na2CO3) solution was added and again incubated in a water bath at 50°C for 5 min. The Folin-Ciocalteau reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. The absorbance of the reaction mixtures was measured at 765 nm using a UV– Vis spectrophotometer (PerkinElmer UV-Vis spectrometer, Lambda 35) against the blank. The experiments were done in triplicate. The absorbance of the extract was compared with a Gallic acid standard curve for estimating the concentration of the total phenol content in the sample. The total phenol content was expressed as mg of Gallic acid equivalents (GAE) per gram of powder on a dry weight

Preparation of standard Gallic acid solution: 200 milligrams of Gallic acid was dissolved into 200ml distilled water, so the concentration of the solution is 0.001

gram/ml or 1000µg/ml. This is called the stock solution. Then serial dilution was performed in order to prepare different concentrated of solution (0 µg/ml, 50 µg/ml, 100 mg/ml, 150 mg/ml, 200mg/ml, 250mg/ml and 300 mg/ml) which is used for preparing calibration curve.

Quantification of total flavonoids: The total flavonoid contents in each extract were determined by the well-known aluminum chloride colorimetric method described by (Viera et al., 2017). In a 20 ml test tube, known volume (1 ml) of the extract was added to a test tube and at zero time, 300 µL of 5% NaNO2 was added. After 5 min, 300 µL of 10% AlCl3 was added and after 6min, 2 ml of 1 M NaOH was added, followed by the addition of 2.4ml of distilled water. The sample absorbance was read at 510 nm using a UV/Vis spectrophotometer (PerkinElmer UV-Vis spectrometer, Lambda 35). The absorbance of the extract was compared with a quercetin standard curve using concentrations of 0 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 and 250 µg/ml for estimating the concentration of flavonoid content in the sample. The flavonoid content was expressed as mg of quercetin equivalents (QE) per gram of powder on a dry weight (DW) basis.

3.3.6. Pretreatment of fabric

To ensure the complete wetting and uniform absorbency of the extracts during padding, the fabric must be undergoing preparatory processes. Scouring treatments were done to cotton fabric to remove foreign materials before imparting finish.

Scouring of the fabric: Scouring of the fabric was done for the removal of natural and added impurities like oils, fats, waxes and other adventitious dirt that may have been added to the fabric during the manufacturing process. The fabric was weighed and soaked before immersion into the scouring bath. The fabric was squeezed thoroughly and treated in a water solution containing 0.5 percent wetting agent, 3 percent caustic soda, 0.5 percent emulsifier and 0.5 percent sequestering agent with a temperature of 98 ℃ with mass liquor ratio (MLR) 1:20 for 90 minutes. After that, the fabric was rinsed with distilled water thoroughly to remove any residues, if left and dried on a flat surface (Sood, 2014).

3.3.7. Application of extract on fabric using a pad-dry-cure method with standard combination

Pad-dry-cure method: The pretreated cotton fabric was treated with 3 g/l and 5 g/l concentration of solanum incanum fruit and red onion peel extract and with their standard combination as described in Table 3.4 along with citric acid (6% w/v) as cross-linking with mass liquor ratio 1:10 (1 gram of fabric in 10 ml of active ingredient solution) using a two-bowl vertical laboratory padder (pressure 2 bar, speed 1.5 m/s, Mathis made in Switzerland) with a two dip and two nip process to get a wet pick up of 95% on the weight of the fabric. After padding, the treated samples were dried at 80℃ for 3 min and cured at 150℃ for 3 min on the lab model curing chamber. Five solutions were prepared for each concentration and designated sample as shown in Table 3.3.

Table 3.3: Fabric treated with different concentrations

Concentration	Type of treatment	Sample code
3 g/l and 5 g/l	25:75 v/v solanum incanum fruit to red onion peel	S1
	50:50 v/v solanum incanum fruit to red onion peel	S2
	75:25 v/v solanum incanum fruit to red onion peel	S3
	100% solanum incanum fruit	S4
	100% red onion peel	S5
	Unfinished	Untreated

3.3.8. Performance evaluation of treated cotton fabrics

Weight Add-On percentage: To determine the weight add-on percentage of the treated sample, solanum incanum (soda apple) fruit and onion peel extracts and their standard combination was applied to the fabric. The weight add-on due to finishing was calculated according to the formula described by (Mondal, Saha, & Environment, 2019)

$$\text{Weight addon}(\%) = \frac{W2-W1}{W1} \times 100 \quad \text{(3. 2)}$$

Where w_1 and w_2 are the weight of untreated and treated fabric respectively. The weight of the fabrics was measured using a digital balance with 0.1 mg accuracy.

Tensile strength test (ES ISO13934): Tensile strength is the ability of the fabric to withstand a load of force usually expressed as kilogram weight, newton or pound weight. The tensile strength of fabric was determined on the paramount universal tensile tester (MESDAN, Italy) using ES ISO 13934 test method. The samples of template size "16 x5" from the warp and "20 x5" from weft directions of the fabric were cut and mounted between the jaws with approximately 1inch of fabric protruding from each side of the jaws at the distance of 3 inches. The instrument was started; the upper jaw was moved in an upward direction until the sample break. The readings were taken from the digital display at sample break. Two readings of the specimen from both the directions (warp and weft) were taken and the average was calculated.

Stiffness test (BS 3356): The fabric stiffness indicates the resistance of the fabric to bending and study the comfort properties of the fabric. The stiffness test method covers the determination of the stiffness of the fabrics by measuring the force required to push a specimen into a slot of predetermined width with a metal blade.

Air permeability (**ES ISO 9237):** The air permeability of a fabric is the volume of air, measured in cubic centimeters passed per second through one square centimeter of the fabric at a pressure of 1 cm of water. Air permeability was tested using air permeability tester (FX 3300, Zurich Switzerland) and the result was noted as cm^3/cm^2. s.

Water absorbency test (AATCC Test Method 79): The water absorbency of both treated and untreated fabrics was evaluated by the water drop method as per AATCC 79-2000 standard. In brief, a drop of distilled water was dispensed from a dropper onto the fabric surface from a distance of 1 cm. Time was recorded until the water drop absorbs completely. A total of four readings were taken for each fabric sample and the mean was calculated.

3.3.9. Biodegradability of finished fabrics

The biodegradability nature of the treated fabric was tested by the burial soil testing method.

Digging soil test (Thilagavathi, Rajendrakumar, & Rajendran, 2005): The soil degradation test was conducted through the method described by (Thilagavathi et al., 2005) with slight modification. Both treated and untreated samples were kept inside the microbial active soil at 10-15 cm depth. The samples were carefully removed from the soil after two weeks and washed with water gently off soil particles, then dried in the sunlight. The degradation (weight loss) of the prescribed samples after two weeks was determined by the following equations.

$$\text{Weight loss}(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{(3. 3)}$$

Where w_1 is the initial weight and w_2 is the after-burial weight

3.3.10. Antibacterial activity testing

Antibacterial testing was done by AATCC test method 100:2004 for a quantitative assessment of the antibacterial effectiveness of the antimicrobial agent against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). Half gram (0.5) of swatches were weighted from the test fabric. The weighted swatches were stacked in 250 ml wide-mouth glass jar with a screw cap followed by sterilization at 121 ℃ for 15 min. To the sterilized glass jar containing the test fabrics, 1ml of bacteria culture solution with a cell concentration of $1x10^6$ CFU/ml and inoculated individual swatches for 18 hours at 37℃. The inoculated samples were incubated for 24 hours at 37℃. After 24 hours incubation, 100 ml of the neutralizing solution prepared by sterilized distilled water was added and shaken vigorously for 1 min. 1 ml of samples were spread on to the agar plate and plates were incubated at 37 ℃ for 24 hours. After incubation, bacterial colonies were counted by a colony counter unit and the antibacterial activity was determined as follows.

% Reduction= [(A-B)/A] ×100 ……. (3. 4)

Where “A” is the number of surviving cells (CFU/ml) for untreated cotton fabric (control), and “B” is the number of surviving bacteria (CFU/ml) for the treated fabrics

3.3.11. Wash durability test

In the present study, the wash durability of the antimicrobial activity of the treated cotton fabric was evaluated at different wash cycles. The treated cotton fabric was washed in a launder –o-meter according to ES ISO 3759 test method with a 5g/l neutral soap solution at 50℃ for 5minutes. The finished fabrics were tested for the retention of antimicrobial activity after 5, 10, 15 launderings by the AATCC-100 test method as described in section 3.2. 10.

4. RESULT AND DISCUSSION

4.1. Determination of Yield Percentage of Plant Extracts

The data presented in Table 4.1 show the percentage yield obtained by the maceration extraction process under the present study. Results revealed that the amount of yield obtained varied between the selected plants, i.e. yield obtained for a methanolic extract of solanum incanum fruit was 13.4 % and onion peel was 17.45%. The percentage yield was determined using the equation described in the methodology part (equation 3.1). Determination of yield of plant extract helps one to estimate the number of plant materials required to generate a required amount of active compound. In the present study, the percentage yield of the methanolic extract of solanum incanum fruit (13.4%) was found to have a better yield as compared to the yield described by Jepkoech& Gakunga (2016) which was 7.8 W/W %. Singh et al. (2017) have been reported that the percentage yield of air-dried onion skin powder using soxhlet extraction technique for 8 hours was 7.46 %. But, the percentage yield of the present study was 17. 45%. This may due to the extraction technique, extraction time, type of solvent used, mass liquor ratio and the demographic area in which the plant material harvested. Lastly, the yield percentage of red onion peel extract was found to be higher as compared to the yield percentage of solanum incanum fruit extract.

Table 4.1: Determination of percentage yield of methanolic extracts of red onion peel and solanum incanum (Sodom apple) fruit.

Name of species	Mean ±SD	Percentage yield	
		Current study	Previous study
Solanum incanum fruit	1.34 ± 0.010	13.4 ± 0. 1	7.46
Red onion peel	1.745 ± 0.057	17.45 ± 0.57	7.8

Key, SD= standard deviation

4.2. Phytochemical Analysis

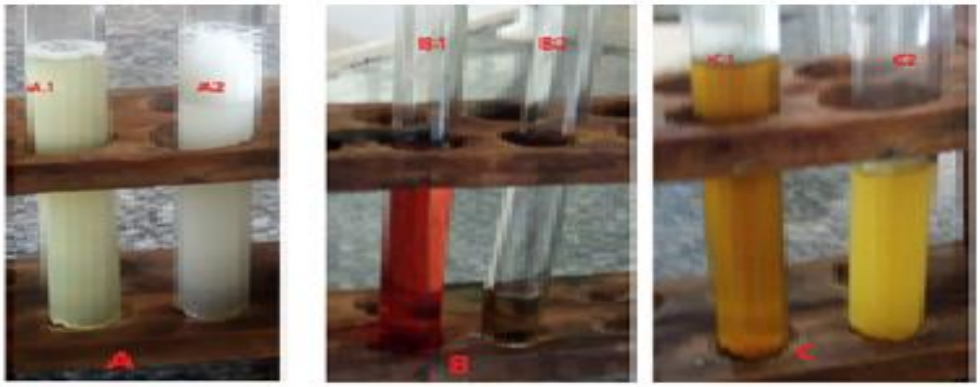
4.2.1. Chemical test analysis

In the present study, the chemical test analysis was conducted to evaluate the presence of active constituents such as alkaloid, flavonoid, steroid, glycoside, protein, resin, quinone, tannin, anthraquinones and phenols. The data presented in Table 4.2, the results of the phytochemical analysis indicate the presence of flavonoids, steroids, glycoside, tannin, protein, quinone, alkaloid, terpenoids, saponins, phenol and the absence of anthraquinone and resin in methanolic fruit extract of solanum incanum. Whereas the phytochemical screening of methanolic extract of onion peel indicates the presence of steroids, terpenoids, alkaloids, flavonoids, quinone, anthraquinones, phenols, saponins, tannin, resin but the absence of protein. As presented in Table 4.2 and Figure 4.1, the present study revealed the presence of saponins, resins, glycosides, tannins, terpenoids, steroids, alkaloids, flavonoids, phenols, quinone, anthraquinones, and proteins.

Table 4.2: Chemical test analysis of a phytochemical component of red onion peel and solanum incanum fruit

Phytochemical	Test	Color change observed	Red onion peel	Solanum incanum fruit
Saponins	Frothing test		+	++
Resins	Sulfuric acid test	Violet	+	-
Glycosides	Alkaline reagent test	Yellow	+	+++
Tannins	Ferric chloride test	Blue-black	+++	++
Terpenoids	Salkowski test	Reddish brown	++	+++
Steroids	Salkowski test	Red	+++	++
Phenol	Ferric chloride test	Blue-black	+++	++
Flavonoids	Alkaline reagent test	Intense yellow	+	++
	Ammonium test	Yellow	+	++
	Aluminum chloride test	Light yellow	-	+
Quinone	Hydrochloric acid test	Yellow	-	+
Anthraquinones	Ammonia test	Red color in ammonia layer	++	-
Protein	Xanthoproteic	Yellow	-	++
Alkaloid	Wagner's reagent test	Reddish/brown precipitate	++	+++

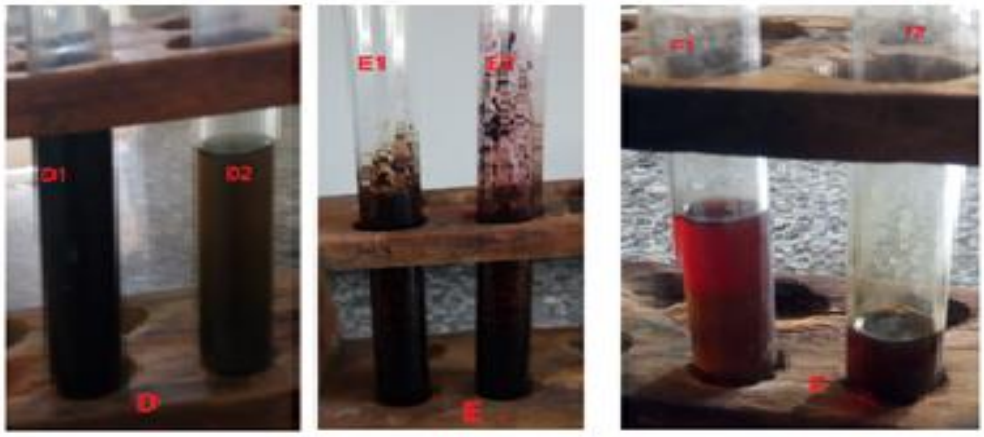
Key (+++) strongly present, (++) moderately present, (+) weakly present and (-) absent



A=Saponin test: Frothing test (Where; A1=Red onion peel; A2 =Solanum incanum fruit)

B=Resin test: Sulfuric acid test (Where; B1=Red onion peel; B2= Solanum incanum fruit)

C=Glycoside: Alkaline Reagent test (Where; C1=Red onion peel; C2 =Solanum incanum fruit)



D=Tannin Test: Ferric chloride Test (D1=Red onion peel; D2=Solanum incanum fruit)

E=Terpenoid Test: Salkowski Test (E1=Red onion peel; E2=Solanum incanum fruit)

F=Steroid Test: Salkowski Test (F1=Red onion peel; F2=Solanum incanum fruit)

G=Phenol Test: Ferric chloride Test (G1=Onion peel;G2=Solanum incanum fruit)
H=I=J=Flavonoid Test ; H=Alkaline Reagent Test; I=Aluminum chloride Test; J=Ammonium Test.
H1=I1=J1=Onion peel; H2=I2=J2=Solanum incanum fruit

K=Protein Test:Xanthoprotic Test (K1=Onion peel; K2=Solanum incanum fruit)
L=Quinone Test: Hydrochloric acid test (L1=Onion peel; Solanum incanum fruit)
M=Anthraquinone Test: Ammonia Test (M1=Onion peel; Solanum incanum fruit)

Figure 4.1: Color change to indicate the various phytochemical test in the methanolic extract of red onion peel and solanum incanum fruit

The present study also supported by the study conducted by Sambo et al. (2000) and Sahle & Okbatinsae (2017) in which solanum incanum fruit extract confirm the presence of saponins, resins, glycosides, tannins, terpenoids, steroids, alkaloids, flavonoids, phenols, quinone, and proteins. The presence of phytochemical constituents like alkaloids, tannin, flavonoids, phenols, saponins and so on in the plants and vegetable peels are known to be responsible for much pharmacological activity as presented by Sambo et al. (2000). The presence of these phytochemicals indicates that S. incanum and onion are a very good source of medicinal plants and the very reason why many traditional medicine practitioners use the fruit and the skin of onion in the treatment of various diseases. Alkaloids responsible for antibacterial and antifungal activities, and hypoglycemic activity as stated by Sambo et al. (2000). Tannins are widely reported to possess antimicrobial and antioxidant activities. Saponins mostly known by its detergent-like properties, commonly used in intracellular histochemistry staining to allow antibody access to intracellular proteins. It has been reported to exhibit hypocholesterolemia, antioxidant, anti-inflammatory, anti-cancer effects. It also effective in antibacterial and antifungal properties. Chandrasekar & Vijayakumar (2017) explained that flavonoid compounds are hydroxylated phenolic substances that occur as a C6-C3 unit linked to an aromatic ring. Their activity is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. The more lipophilic flavonoids also disrupt the microbial membrane. Many researchers have been reported that flavonoids possess antioxidant, anticancer and anti-inflammatory. In addition, flavonoids possess antimicrobial activities, inhibit lipid peroxidation, platelet aggregation, and capillary permeability as described by Sambo et al. (2000). In conclusion, phytochemical screening of medicinal plant fruit and the vegetable peel is very important in identifying new sources of therapeutically and industrially important compounds as described by Sahle & Okbatinsae (2017). It is imperative to initiate urgent steps for a screening of plants and vegetables for secondary metabolites.

4.2.2. Fourier Transform Infrared Spectroscopy Analysis

The FT-IR spectrum of methanolic extract of solanum incanum fruit and red onion peel are given in Figures 4.2 and 4.3. The data on the peak values and the probable functional groups (obtained by FT-IR analysis) present in the methanolic extract of the fruit of solanum incanum plant and in the peel of onion vegetables were presented in Table 4.3. The region of infrared radiation helps to identify the functional group of the active component present in the extracts based on the peak value of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak's ratio.

Table 4.3: FT-IR spectral peak value, bond and functional groups obtained for a methanolic extract of red onion peel and solanum incanum fruit

Methanolic Extract					
Solanum incanum fruit			Red onion peel		
Peak values	Bond	Functional group	Peak values	Bond	Functional group
3009.6	C-H-Stretch	alkene	3793.6	O-H-Stretch, Free hydroxyl	Alcohol, phenol
2852.8	O-H-Stretch	Carboxylic acid	2222.2	C (triple bond) N stretch	Nitrile
2923.2	C-H-Stretch	Alkane	2161.6	C (triple bond) C stretch	Alkyne
1745.6	C=O-Stretch	Carbonyl compounds, esters, aldehyde	910.4	O-H-bend	Carboxylic acid
1460.8	C-C-Stretch (in-ring)	Aromatic ring	1393.6	C-C-Stretch (in-ring)	Aromatic
1163.2	C-N-Stretch	Aliphatic amine	2001.6		Aromatic overtone
1032	C-O-Stretch	Alcohol	1966.4		Aromatic ring

The results of FTIR analysis confirmed the presence of alcohol, alkene, alkane, amine, alkane, nitrile, ester, alkyne, aldehyde and aromatic compound. Harborne (1998) suggested that the presence of the functional group's alkanes, alkenes, aromatic, alcohols and phenols, aldehydes and ketones, esters and lactones, carboxylic acids,

amine, cyanides, and isocyanates from IR spectra analysis confirms the presence of phytochemical constituent. The absorbance bands analysis in the bio-reduction was observed in the region 400-4000 cm-1 are 1032, 1163.2, 1460.8, 1745.6, 2923.2, 2852.8 and 3009.6 cm-1 for a methanolic extract of solanum incanum (Sodom apple) fruit. The major peak observed in the methanolic extract of solanum incanum fruit is 1032, 1745.6, 2852.8 and 1460.8 that indicating the presence of C-O-Stretch alcohol, C=O-Stretch carbonyl compounds and esters, O-H-Stretch carboxylic acid and C-C-Stretch (in-ring) aromatic ring. Whereas, the absorbance bands analysis in the bio-reduction was observed in the region 4000-400 cm-1 for a methanolic extract of red onion peel are 3793.6, 2222.2, 2161.6, 2001.6, 1966.4, 1393.6 and 910.4 cm-1 presented in Table 5. The major peak observed in the methanolic extract of red onion peel was 3793.6, 910.4 and 1393.6 that are indicating the presence of O-H-stretch alcohol, phenol, C-C-stretch aromatic compound, and O-H-bend carboxylic acid respectively.

Ashokkumar et al. (2014) carried out the FT-IR spectra analysis of leaf of four medicinal plant such as Phyllanthus amarus, Phyllanthus maderaspatensis (Family: Euphorbiaceae), Senna auriculata (Family: Caesalpiniaceae), Solanum torvum (Family: Solanaceae) and reported the presence of characteristic functional groups alcohol, carboxylic acid, carbonyl compounds, alkane, aromatic ring.

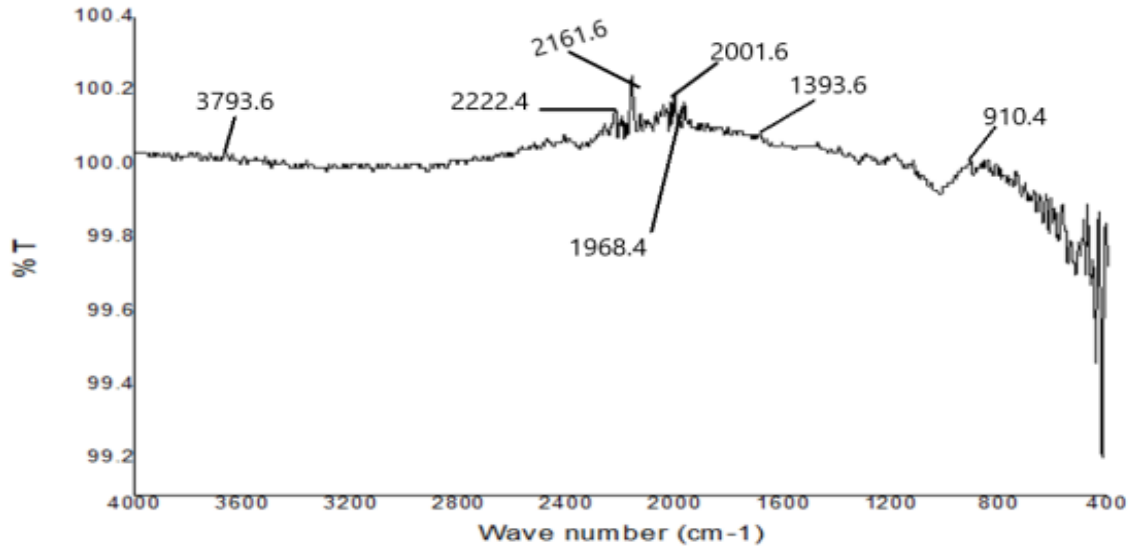


Figure 4.2: FT-IR spectrum of methanolic extract of red onion peel

In general, the FT-IR analysis of the methanolic extract of solanum incanum fruit and red onion peel revealed the presence of alkene, carboxylic acid, alcohol, carbonyl compounds, ester, alkane, aromatic ring compound, aliphatic amine. This result supports the presence of flavonoids, phenols, alkaloids, glycosides, tannin, saponins and steroids.

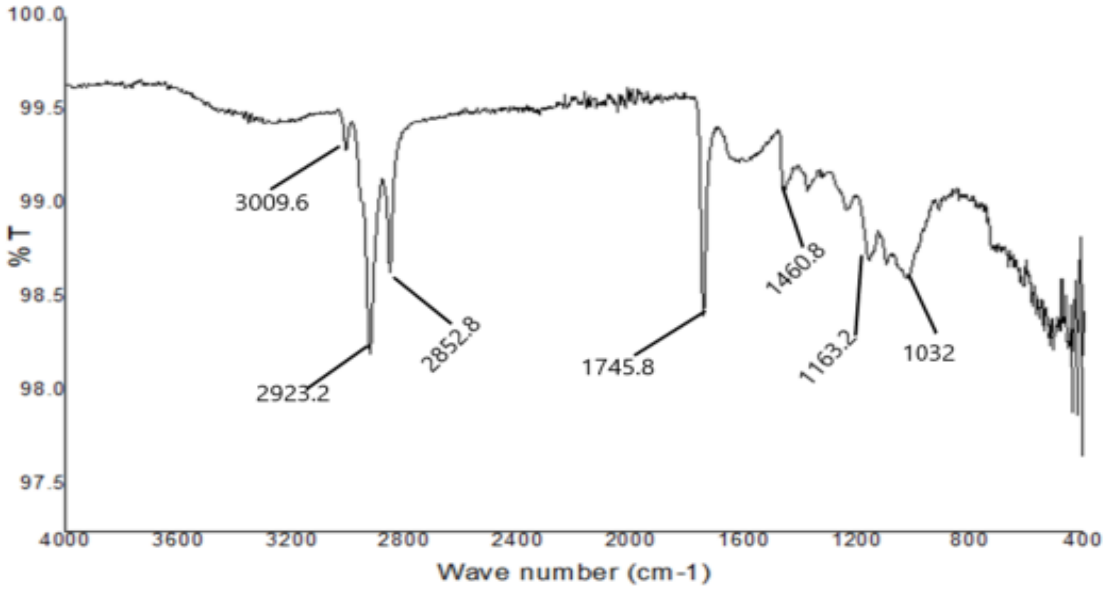


Figure 4.3: FT-IR spectrum of methanolic extract of solanum incanum fruit

4.2.3. Quantification of total polyphenol and total flavonoid content

Total polyphenol content

The amount of total phenol content in each extract was determined by Folin-Ciocalteu's reagent described in the method section using Gallic acid as the standard. The absorbance values obtained at different concentrations of Gallic acid presented in Table 4.4 were used for the construction of the calibration curve as presented in appendix 4.2 (Figure 4.2A).). The levels of total flavonoid were expressed in terms of Gallic acid equivalent (GAE), determined by the well-known method described by Folin-Ciocalteu's reagent and quantified from the equation of regression line: $A = 0.006C + 0.045$ with $R^2 = 0.969$ from appendix 4.2 (Figure 4.2A) where A= mean absorbance, C= concentration in mg/L. The total phenol content can be determined using the

formula $TPC(\frac{mgGAE}{g}) = \frac{C*V}{m}$ where, C=Concentration of gallic acid in mg/L, V=Volume of a sample taken (ml), TPC= Total phenol content in mg of Gallic acid equivalents and m=dry mass of a sample.

The level of the total phenol contents of the methanolic extracts of solanum incanum fruit and red onion peel were presented in the table below (Table 4.5). It appeared that the methanolic extract of red onion peel had the highest phenol content compounds (2.657 ± 0.00078 mg GAE/g DW) than that of the same extract of solanum incanum fruit (1.415 ± 0.00136 mg GAE/g DW). Ifesan (2017), reported that the total phenol of ethanolic extract of red onion skin was 714.33 ± 66.09 µg GAE/ml from 1000 µg/ml concentration. The total phenol content in the present study was 2657 ± 0.78 µg GAE/g DW from 1000 µg/ml was higher than that of the total phenol content has been reported by Ifesan (2017) which was 714.33 ± 66.09µg GAE/ml from 1000 µg/ml concentration of the sample. This may due to the type of solvent used, solvent strength, the technique of extraction and time of extraction. The quantification result of the phenolic compound confirms the qualitative test of phenol as described in table 4.5 and Figure 4.1. As discussed in Table 4.2, the color change observed for a phenolic test in red onion peel extract is confirmed strongly in the presence of a phenolic compound in red onion peel extract than the presence of a phenolic compound in solanum incanum fruit extract (moderately present). This result reveals that the quantification result from the UV-Vis spectrometer supports the qualitative test.

Table 4.4: Data for calibration curve construction using standard Gallic acid for total phenol determination

Standard Gallic acid in mg/L	Standard Gallic acid in ml	Folin-Ciocalteau reagent in ml	7.5 %Na_2CO_3 in ml	Mean absorbance @ 765nm
0	0	5	4	0
50	1	5	4	0.484
100	1	5	4	0.872
150	1	5	4	1.363
200	1	5	4	1.578
250	1	5	4	1.73
300	1	5	4	2.005

Table 4.5: Total phenol content of methanolic extract of red onion peel and solanum incanum fruit

Extracts (µg/ml)	Total phenol content (mg GAE/g dry sample)			
	Red onion peel		Solanum incanum fruit	
	Current study	Previous study	Current study	Previous study
1000	2.657± 0.00078	0.71433± 0.06609	1.415 ± 0.00136	No studied

Key, **GAE= Gallic Acid Equivalent (conventional unit for phenolic compound)

All values are mean of triplicate experiments

Total Flavonoid Content

The amount of total flavonoid content in each extract was determined by the aluminum chloride colorimetric method described in the method section using quercetin acid as the standard. The absorbance values obtained at different concentrations of quercetin indicated in Table 4.6 was used for the construction of a calibration curve from appendix 4.2 (Figure 4.2B). The levels of total flavonoid were expressed in terms of quercetin equivalent (QUE), determined by the well-known aluminum chloride colorimetric method described by Viera et al. (2017)and quantified from the equation of regression line: $A = 0.0007C + 0.026$ with $R^2 = 0.935$ from appendix (Figure 4.2B) where A= mean absorbance, C= concentration quercetin in mg/L. The total flavonoid content of the extracts can be determined using the formula $\text{TFC}\left(\frac{\text{mgQUE}}{\text{g}}\right) = \frac{C*V}{m}$ where C=Concentration of quercetin in mg/L of quercetin equivalence, V=Volume of a sample taken (ml), m=dry weight of the sample (g) and TFC=Total flavonoid content in mg of quercetin equivalence. The level of the total flavonoid contents of the methanolic extracts of solanum incanum fruit and red onion peel are presented in the table below (Table 4.7). It appeared that the methanolic extract of solanum incanum fruit had the highest flavonoid content compounds (4.949± 0.0067 mg QUE/g DW) than that of the same extract of red onion peel (2.866 ± 0.0294 mg QUE/g DW). Ifesan (2017), reported that the total flavonoid of ethanolic extract of red onion skin was 177.33 µg QUE/ml from 1000 µg/ml concentration. The total flavonoid

content in the present study which was 2866 ± 29.4 µg QUE/g DW from 1000 µg/ml was higher than that of the total flavonoid content has been reported by Ifesan (2017) which was 177.33 µg QUE/ml from 1000 µg/ml concentration of a sample. This may due to the type of solvent used, solvent strength, the technique of extraction and time of extraction. The risk of heart disease, inhibit the initiation, promotion, and progression of tumors can be reduced by flavonoid compounds. The quantification result of the phenolic compound confirms the qualitative test of phenol as described in Table 4.2 and Figure 4.1. As discussed in Table 4.2, the color change observed for the flavonoid test in solanum incanum fruit extract is confirmed moderately presence of flavonoid compound in solanum incanum fruit extract than the presence of flavonoid compound in red onion peel extract (weakly present). This result reveals that the quantification result from the UV-Vis spectrometer supports the qualitative test.

Table 4.6: Data for calibration curve construction using standard quercetin acid for total flavonoid determination

standard quercetin in mg/l	Standard quercetin in ml	10 % AlCl3 in ml	5% NaNO2	1M NaOH in ml	Distilled water in ml	Mean absorbance @ 510 nm
0	0	0.3	0.3	2	3.4	0
50	1	0.3	0.3	2	2.4	0.087
100	1	0.3	0.3	2	2.4	0.105
150	1	0.3	0.3	2	2.4	0.131
200	1	0.3	0.3	2	2.4	0.164
250	1	0.3	0.3	2	2.4	0.194

Table 4.7: Total flavonoid contents of methanolic extract of solanum incanum and red onion peel

Extracts (µg/ml)	Total flavonoid content (mg QUE/g dry sample)			
	Red onion peel		Solanum incanum fruit	
	Current study	Previous study	Current study	Previous study
1000	2.866 ± 0.0294	0.17733	4.949 ± 0.0067	No studied

Key, **QUE=Quercetin Equivalence (the conventional unit for flavonoid compound)

**1000 µg=1 mg, 1000mg=1 g. All values are mean of triplicate experiments

4.3. Physical Properties of Treated Cotton Fabric

In the present study, 100 % bleached cotton fabric was treated with solanum incanum fruit and red onion peel extract in the presence of citric acid as a cross-linking agent. The data depicted in Figure 4.4 and Table 4.3A in appendix 4.3, there was a 4.76% change in the bending length of the treated fabric as compared to the untreated one and the bending length is directly related to the flexural rigidity of the fabric. Even if the bending length of S5 (0:100) with a concentration of 5 g/l is high but it is not significant to affect the handle of fabric when wore on human skin. Thus, the flexibility of the fabric is not changed too much even after the treatment process. (Mukthy, Azim, & Technology, 2014) studied the effect crosslinking agent on bending length and observed that the bending length of the treated cotton fabric increased in both warp and weft direction and attributed such an increase in the formation of covalent bonds that held the cellulose molecules together. Although studies revealed that the presence of resin cross-linking agent and herb extract, can cause an increase of the bending length of cotton fabric.

(Mortazavi & Esmailzadeh, 2004) also studied the influence of the cross-linking agent on the bending length and revealed that the bending length has magnified with an increase in resin concentration, due to the cross-linking of cellulose. They also revealed that under stress, the hydrogen bonds between adjacent cellulose chains can break allowing the chain to slip each other. But after cross-linking of cellulose, some

hydrogen bonds get converted to covalent bonds, therefore, bending length increase. The result published by Mortazavi & Esmailzadeh (2004) and Mukthy et al. (2014) support the result obtained in the present study.

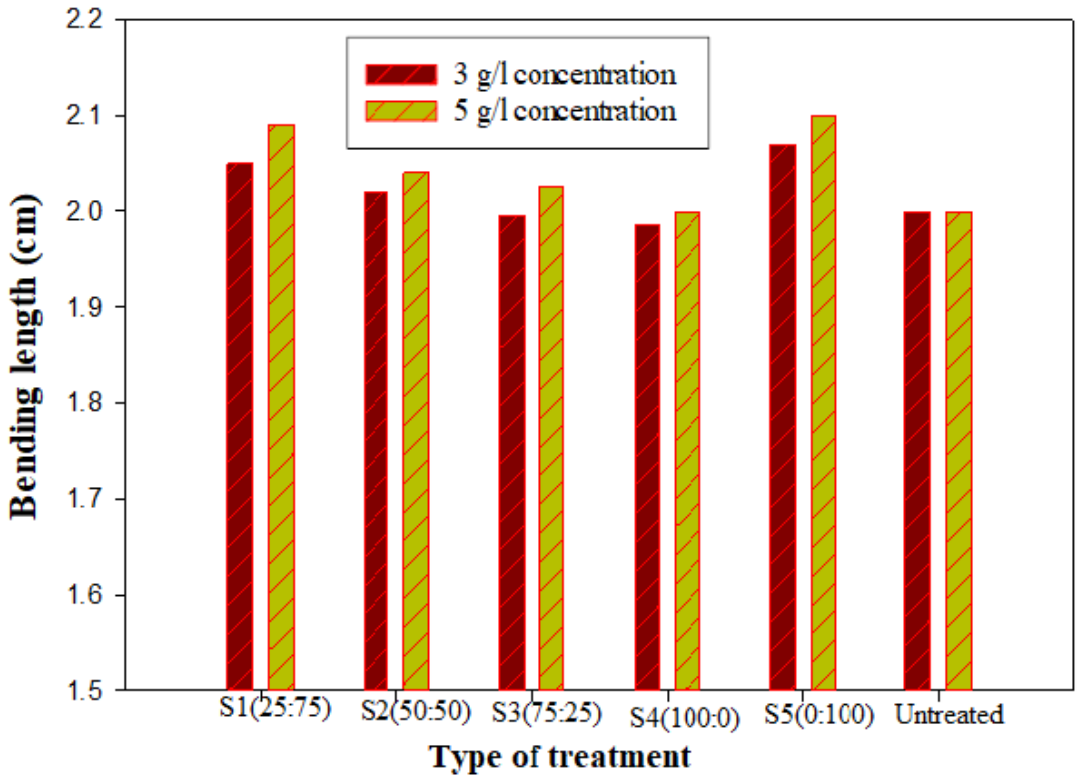


Figure 4.4: Effect of an antibacterial agent on bending length of tested fabric

Air permeability is an indication of the rate of airflow through the fabric. By increasing the concentration of the antimicrobial agent, from 3 to 5 g/l, applied on the cotton fabric, the thickness of the fabric was increased. As a result, the air permeability was slightly decreased as described by Mondal et al. (2019). The test result presented in Figure 4.5 and Table 4.2C in appendix 4.3 provided that, solanum incanum fruit and red onion peel extract finished fabrics had lower air permeability than that of unfinished fabrics. This is because solanum incanum fruit and red onion peel extract treatments fill the pore of the fabric. As compared to the air permeability of pure extract finished fabric, fabric treated with pure solanum incanum fruit extract (S4) had better air permeability than that of pure red onion peel extract finished fabric (S5). This is due to the better affinity of red onion peel extract towards the fabric than solanum incanum fruit extract

and the coating layer thickness was larger in the case of red onion peel extract-treated fabric compared to solanum incanum fruit extract treated fabrics. For combinatorial treated fabrics, fabric treated with 75% (S3) of solanum incanum fruit extract had well air permeability compared to fabric finished with 25% (S1) and 50 % (S2) solanum incanum fruit for both concentrations (3 g/l and 5 g/l). According to El-Shafei et al. (2017), air permeability should be ultimately tested to decide the comfort properties of the fabric to the wearer. When the amount of air permeability decreases then discomfort arises.

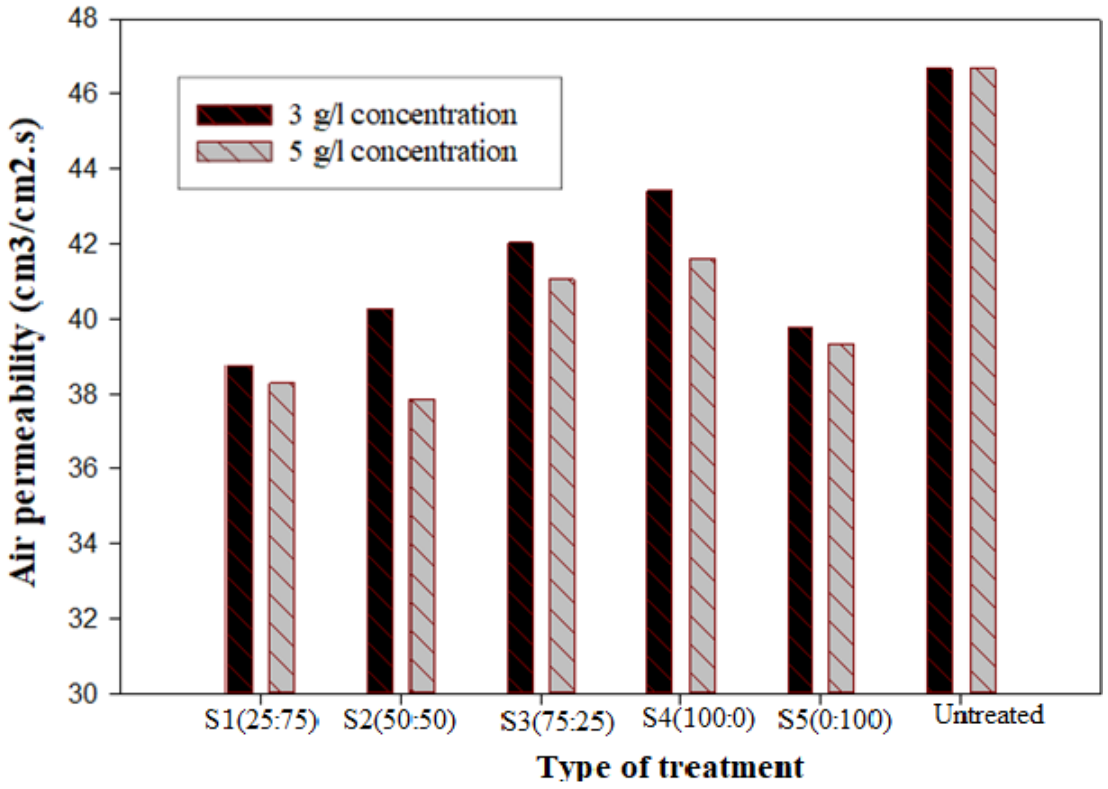


Figure 4.5: Effect of an antibacterial agent on air permeability

The breathability of the fabric was not changed significantly due to the finishing of peel and fruit extracts. The result of the present study was similar to the finding of El-Shafei et al. (2017) and Mondal et al. (2019) who reported that applying herb extract on the fabric surface did not much significantly alert the air permeability of the fabric.

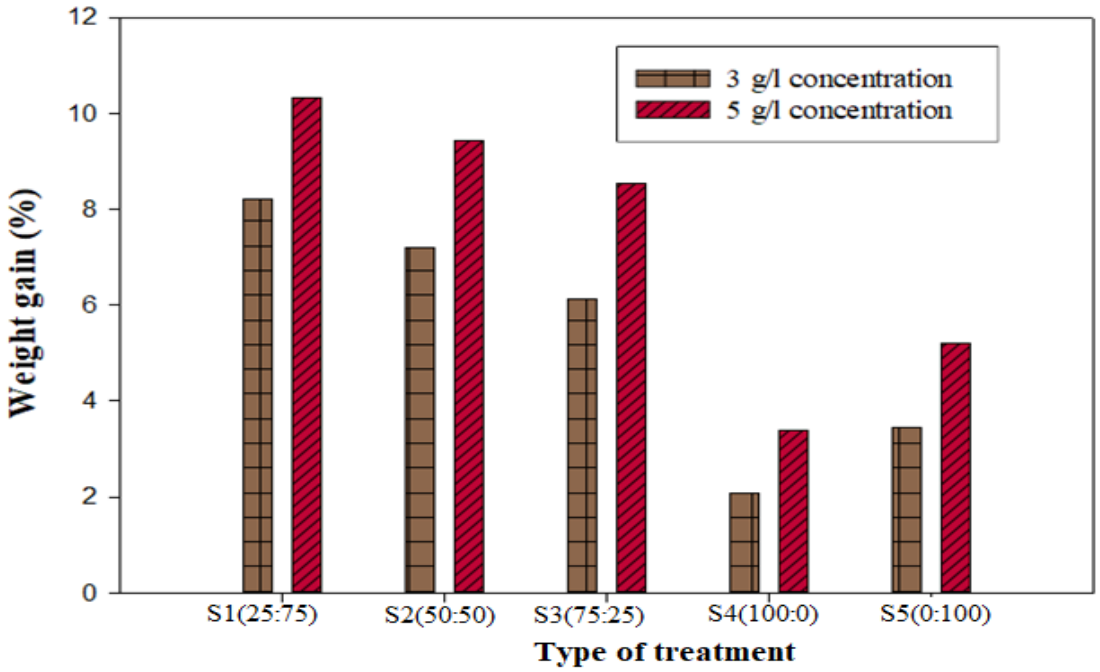


Figure 4.6: Effect of an antibacterial agent on weight adds the test fabric

Data depicted in Figure 4.6 and Table 4.3B in appendix 4.3, the weights add on percentage increase as the concentration of the antimicrobial agent increase, since more extract attached to the fabric during finishing. The weight gain percentage of the combinatorial finished fabric was higher than that of the pure extract fished fabric since the degree of affinity of the extract towards the fabric is different. As comparing the weight gain percentage of the fabric treated with pure extracts, the fabric finished with pure red onion peel had better weight gain percentage than that of fabric treated with pure solanum incanum fruit, in the same concentration. This is due to the high degree affinity of red onion peel extract to the cotton fabric. That is why test fabric treated with a binary ratio of 25:75 (solanum incanum fruit to red onion peel) for both concentrations (3 and 5 g/l) had better weight gain percentage than any other combinatorial ratio treated fabric. The present finding is in accordance with Mondal et al. (2019) report that weight add-on percentage increased with an increase in the concentration of chitosan and aloe-Vera extracts. The present finding also supported by Sood (2014) who report that application of different herb extract on the cotton fabric, results in the increase of weight gain percentage.

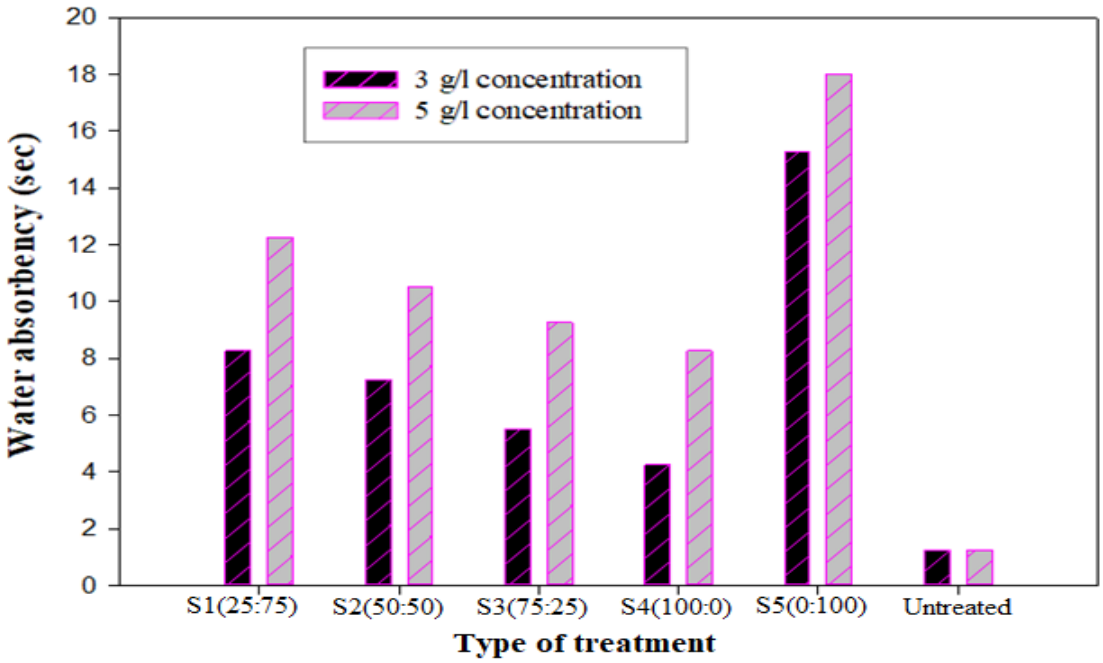


Figure 4.7: Effect of the antibacterial agent on water absorbency of test fabric

Absorbency (or wetting) defined as the time in seconds in which a drop of distilled water to sink into the fabric. The extent of hydrophilicity is inversely proportional to the time taken by the water to sink into the fabric completely. According to Sood (2014), if the time to sink water droplet exceeds 20 seconds, the fabric is considered unwettable. Water absorbency of the fabric treated with different combinations extracts ratio has presented in Figure 4.7 and Table 4.2D in appendix 4.3. Fabric treated with pure red onion peel extract shows water repellency. This could be due to, the hydrophobic nature of the extract, which forms a thin coating of film on the surface of the fabric. As comparing the water absorbency of fabric treated with combinatorial extract ratio, the fabric treated with 75% of red onion peel extracts (sample1) for both concentration (3 and 5g/l) had greater water repellency than any other type of treatment. This shows that the hydrophobic nature of the red onion peels is greater than that of the hydrophobic nature of solanum incanum fruit. In general, the absorbency values indicate that the water-absorbing nature of the fabric was not altered much. The results of the present finding also supported by the result of the previous author of Mondal et al. (2019), Sathianarayanan et al. (2010) and Sood (2014).

As presented in figure 4.8 and Table 4.3E in appendix 4.3, the maximum strength loss happened the fabric treated with pure red onion peel extract in the warp and weft direction of the fabric for both concentrations. The maximum percentage loss of strength was 44% in the warp and 24.24 % in weft direction at 5 g/l concentration and 20 % in the warp and 15.15% in weft direction at 3 g/l concentration. Whereas the minimum strength loss has happened, the fabric treated with pure solanum incanum fruit extract in warp and weft direction for both concentrations. The minimum percentage loss of strength was 9.85% in the weft and 12% in the warp direction for 3 g/l concentration and 20% and 13.63% in warp and weft direction for 5 g/l concentration respectively. The loss of strength is mainly due to the stiffening of the molecular backbone after cross-link information Ali et al. (2014).

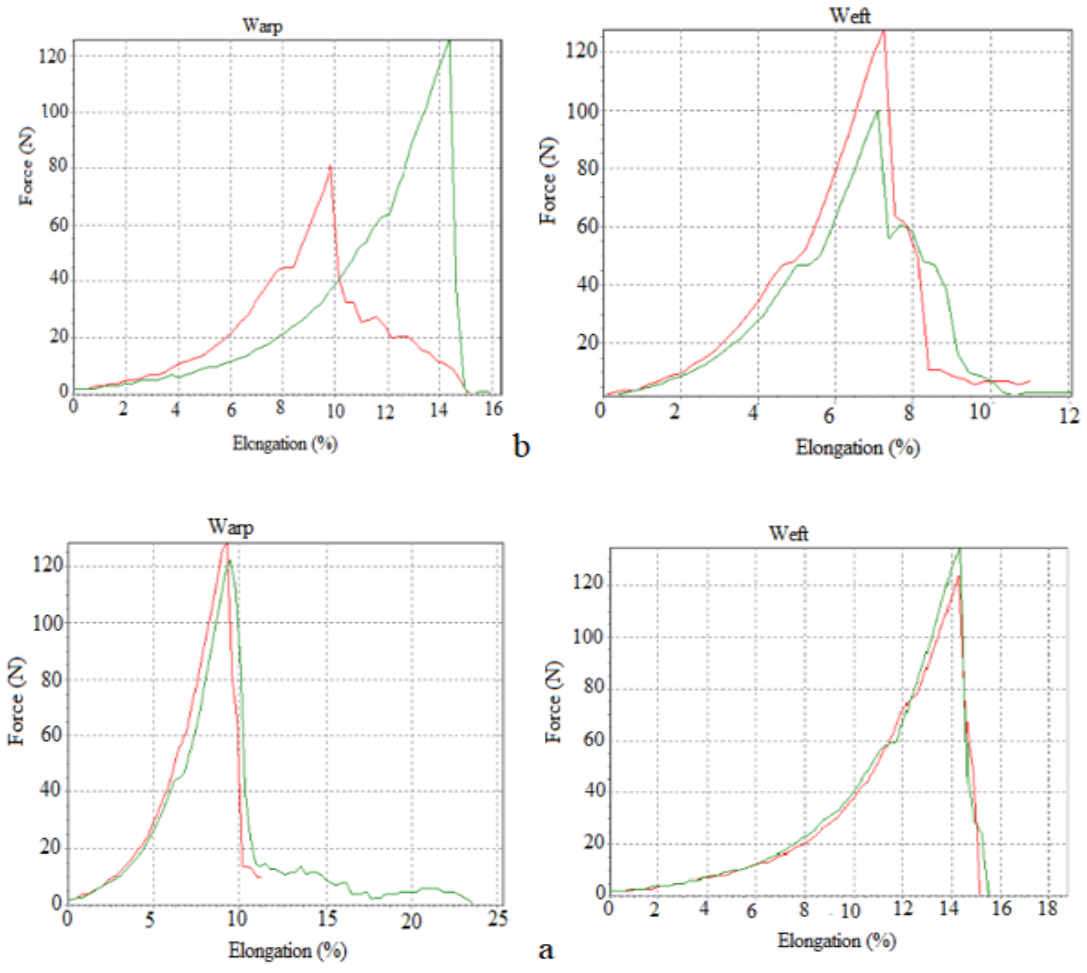


Figure 4.8: Test of fabric traction with Mesdan Lab strength tester (a) untreated (b) treated

Such strength loss of citric acid-treated cotton is also attributed to acid-catalyzed depolymerization of cellulose molecules. In the other view, the reason why the strength loss happened was probably the result of hydrolysis of cotton cellulose macromolecules during treatment, in the presence of citric acid as described by Mondal et al. (2019).

According to (Tsuji, 1971), cotton has a large degree of polymerization and crystallinity and rather narrow, restricted non-crystalline region. Therefore, the mobility molecular segment in the non-crystalline region is restricted. When the tension has given to it, the even distribution of tensile stress is restrained and the stress concentration is a point to occur. If cross-linking has formed in the non-crystalline region, the mobility of the molecular segment is more restricted and stress concentration increased, thus a decrease of tensile strength has caused. (Sunder & Nalankilli, 2012) studied on the polyfunctional finishes on cotton textiles stated that the decrease tensile strength may be attributed to the presence of a cross-linking agent. The decrease tensile strength could be attributed to the formation of intermolecular and intramolecular cross-links which reduce the possibility of equalizing the stress distribution, causing a reduction in the capacity to withstand the load, as well as prevent the movement of the fiber molecules causing severe tensile strength loss as reported by Sunder & Nalankilli (2012). With increasing in the antimicrobial agent concentration, the tensile strength of the treated fabric decreases in all cases of treatment. However, the loss in strength was not much significant. The results of Ali et al. (2014); Sathianarayanan et al. (2010); Sood (2014) and Nalankilli& Tadesse (2018) support the result of the present finding.

4.4. Antibacterial Activity of Treated Fabric (AATCC Test Method 100-2004)

The bacterial resistance of the fabric finished with pure solanum incanum fruit and red onion peel extract and their combinations with two different concentrations i.e. 3 g/l and 5 g/l by pad-dry-cure method, the growth of E. coli (Gram-negative bacteria) and S. aureus (Gram-positive bacteria) was counted quantitatively by the standard test method AATCC 100. The efficacy of solanum incanum fruit and red onion peel and their standard combinations has compared against the control sample. In the colony

count test, Table 4.3H in appendix 4.3 shows that the bacterial reduction of the fabric treated with solanum incanum fruit and red onion peel extracts and their combination as compared with the control sample against gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (E. coli).

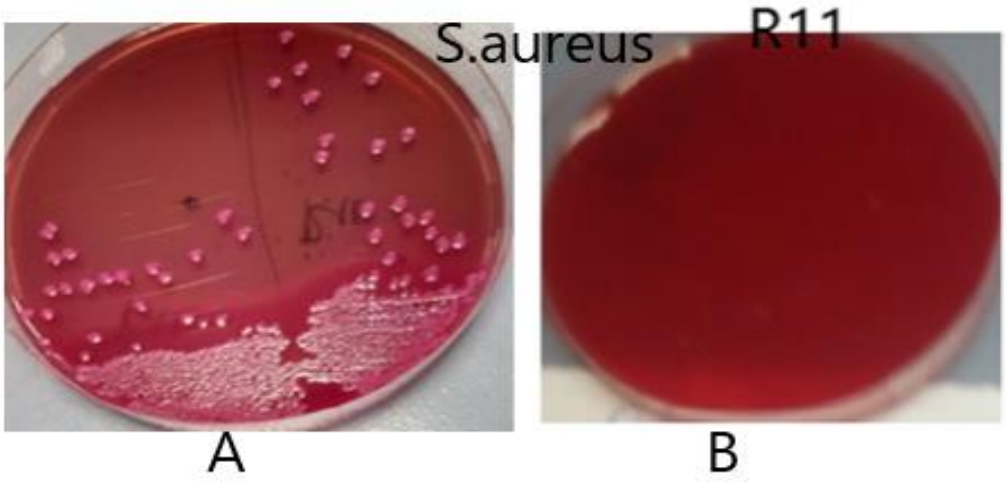


Figure 4.9: Antibacterial activity of cotton fabric by AATCC 100-2004 method against S.aureus; A=Control, B=Cotton fabric treated with 50:50 combination of solanum incanum fruit and red onion peel extract at 5 g/l concentration

As presented in Figure 4.10 and Table 4.3H in appendix 4.3, after treating the cotton fabric by a pad-dry-cure method with 3 g/l and 5 g/l concentration of red onion peel separately, percentage reduction values were 97.17 % and 97.67 % for E. coli and 97.50% and 98.00% for S. aureus, respectively. Data (Table 4.3H) also revealed that when solanum incanum fruit extract was applied by the pad-dry-cure method, percentage reduction values were 97.83 % and 98.0% for E. coli and 97.92 % and 98.83% for S. aureus. Data (Table 4.3H) also depicted the bacterial reduction against various combinations of solanum incanum fruit and red onion peel extracts. It was found that 50:50 v/v combinations of solanum incanum fruit and red onion peel extract had better bacterial reduction i.e. 99.63% for E. coli and 99.83% for S. aureus with 3 g/l concentration followed by 75:25 v/v (99.0% for E. coli and 99.2 for S. aureus) and 25:75 v/v (98.08% for E. coli and 98.42% for S. aureus). With 5 g/l concentration of the same extract i.e. combination of solanum incanum fruit and red onion peel, the

bacterial reduction values increased to 99.92%, 99.42% and 98.92 % using 50:50, 75:25 and 25:75 v/v standard combinations on cotton fabric for E. coli bacteria. The bacterial reduction of the fabric treated with combined extract of solanum incanum fruit and red onion peel was found better than the fabric treated with alone. This is maybe due to the cumulative effect and different chemical composition of solanum incanum and red onion peel extract in combinatorial treatment. As comparing the bacterial reduction of fabric treated with pure extract, the fabric treated with pure solanum incanum fruit extract had maximum bacterial reduction than that of red onion peel extract against S. aureus and E. coli test bacteria. This is due to the chemical composition of solanum incanum fruit extract i.e. the presence of a high number of flavonoids, which has known to exhibit a remarkable degree of antibacterial activity. In accordance, Alkhalifah & Science(2016) found that the methanolic extract of solanum incanum has better activity on E. coli and S. aureus. According to Ortiz& Technology (2015), onion peel extract has strong antibacterial activity against S. aureus and E. coli.

According to Sood (2014), the major differences between the Gram-positive and Gram-negative bacteria arises from the chemical composition of the cell wall of the bacteria. The content of lipopolysaccharide contained in the cell wall of Gram-negative bacteria is very important and the low-efficiency antibacterial agents have more difficulties in crossing the cell wall of Gram-negative bacteria in comparison to the cell wall of Gram-positive bacteria.

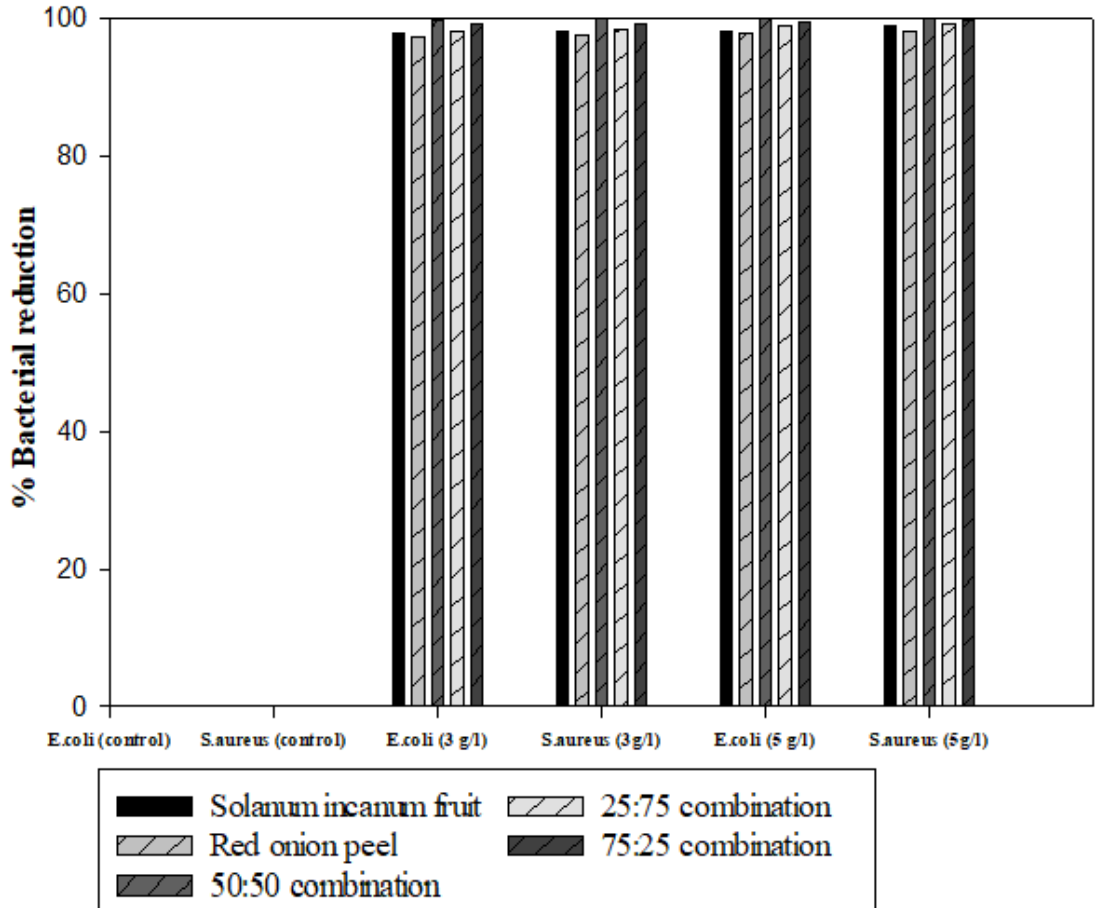


Figure 4.10: Antibacterial activity of antimicrobial finished cotton fabric

So higher concentrations of antibacterial agents are usually required to inhibit Gram-negative bacteria. But in the present study, all types of treated fabric exhibited very good efficacy against both Gram-positive S. aureus and Gram-negative E. coli, which indicate that the plant extracts of solanum incanum fruit and vegetable extracts of red onion peel were effective against Gram-negative and Gram-positive bacteria. The value of percentage reduction indicates that both the extracts and their combination not only prevent the growth of bacteria but also kills the bacteria (bactericides). The present finding also suggested by Sathianarayanan et al. (2010) who report that the application of tulsi leaf and pomegranate fruit extract on the cotton fabric, the percent reduction of bacteria was 99.9 % against S. aureus for both herb extracts applied indirect method (paddy-dry-cure).

According to Singh et al. (2017) study, the efficacy of 50:50 combinations of pomegranate and onion peel extract had better efficacy than of the efficacy of extracts in 25:75 and 75:25 combinations of solanum incanum fruit and red onion peel. They also report that the cotton fabric finished with a different combination of pomegranate and onion peel extracts i.e. 50:50, 25:75 and 75:25 had shown better efficacy against bacterial growth as compared to pure pomegranate and onion peel extracts. In general, the present result supported by Ali et al. (2014), Mondal et al. (2019) and Singh et al. (2017).

To sum up, the methanolic extracts exhibited better antibacterial activity against gram-positive bacteria than gram-negative bacteria. The observations differentiate the existence of major differences in the outer layers of gram-positive and gram-negative bacteria. The gram-negative bacteria characterized by an outer membrane and a unique periplasmic space which not found in gram-positive bacteria. The resistance of gram-negative bacteria towards antibacterial agents was related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules (Varier, Milton, Arulvasu, Gajendran, & Research, 2013).

The membrane is also associated with the enzymes in the periplasmic space which were capable of breaking down the molecules introduced from outside (Varier et al., 2013). However, the Gram-positive bacteria do not possess such outer membrane and cell wall structures (Varier et al., 2013). In the case of gram-negative bacterial strains, a thin layer of peptidoglycan in the cell may fail to provide an active site for the binding of the bioactive compound leading to a reduced inhibitory effect (Varier et al., 2013). An important characteristic of plant extracts and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Chandrasekar & Vijayakumar, 2017). According to Chandrasekar & Vijayakumar (2017) explanation, extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death.

4.5. Wash Durability Test

As data depicted in figure 4.11 and Table 4.3G in appendix 4.3, the bacterial reduction of the fabric treated with 50:50 combinations of solanum incanum fruit and red onion peel extract at 5 g/l concentration shows a slight change after 15 wash cycle for both Gram-positive (S. aureus) and Gram-negative (E. coli) test bacteria. Whereas the bacterial reduction of the fabric treated with pure solanum incanum fruit and red onion peel had a significant change after a 15-wash cycle at the same concentration. The decrease in antibacterial activity may be attributed to the slow removal of the extract, due to the breakdown of cross-links between the finishing agent and the cellulose material which explains the bonding between the finishing agent and the fabric structure. As comparing the wash durability of fabric treated with pure extract, the fabric treated with pure solanum incanum fruit extract had better efficacy than that of red onion peel extract against S. aureus and E. coli test bacteria. Data also presented in the figure below the wash durability of the synthetic antimicrobial agent (silver nitrate) decrease after 10 wash cycle. Fabric treated with 50:50 combinations of solanum incanum fruit and onion peel extract had better wash durability than fabric treated with the synthetic antimicrobial agent (silver nitrate). This was due to the leaching property of synthetic agents.

These results are supported by the study conducted by Unango et al. (2019) who report on the investigation of biologically active natural compounds on cotton fabrics as an antibacterial textile finishing. Thus, the wash durability test of the present study on antibacterial activity also suggested the study conducted by Singh et al. (2017) and Nalankilli &Tadesse (2018).

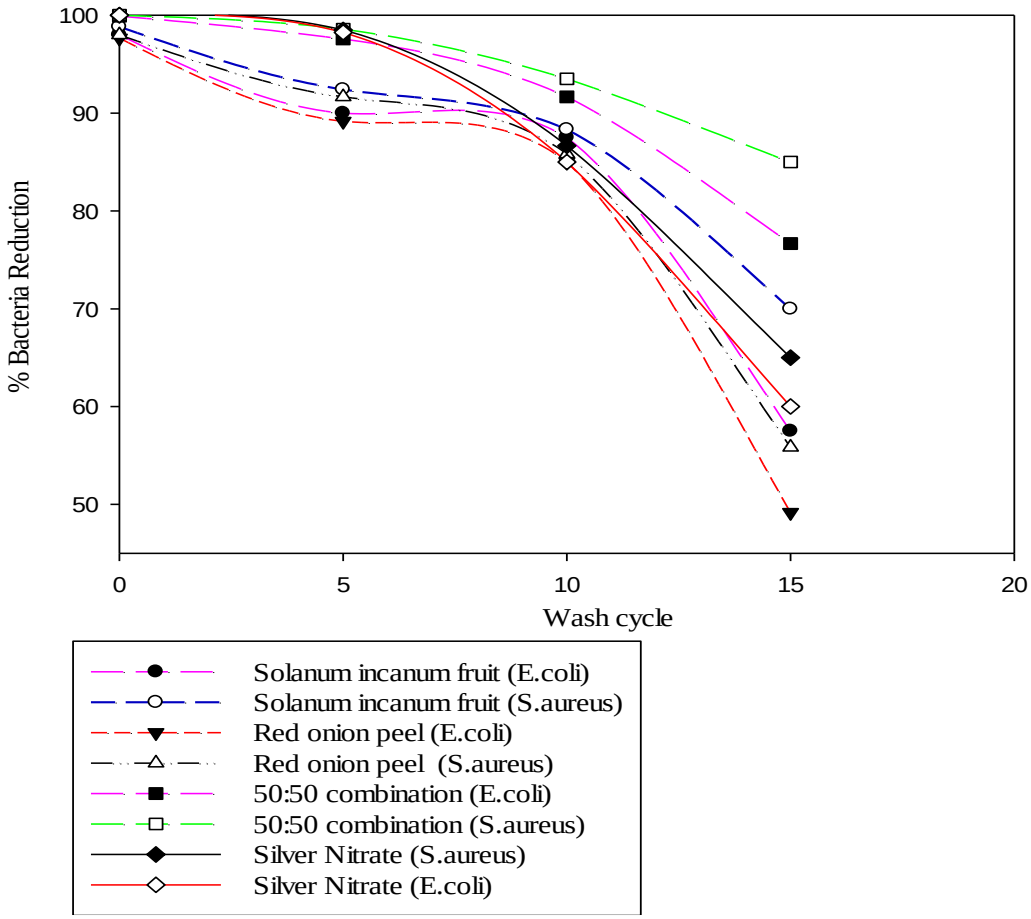


Figure 4.11: Effect of wash durability on antibacterial activity of cotton fabric against S. aureus and E. coli

4.6. Biodegradability of the Fabric

Digging soil test

The weight loss of the fabric due to digging soil test after two weeks was calculated by using the equation stated under section 3.2.9 (equation 3.3). As stated in Figure 4.12 and Table 4.3F from the appendix 4.3, the maximum weight loss because of soil degradation was that of the untreated fabric, this is because microorganisms in the soil attacked the untreated cotton fabric quickly, in the absence of any treatment to inhibit them.

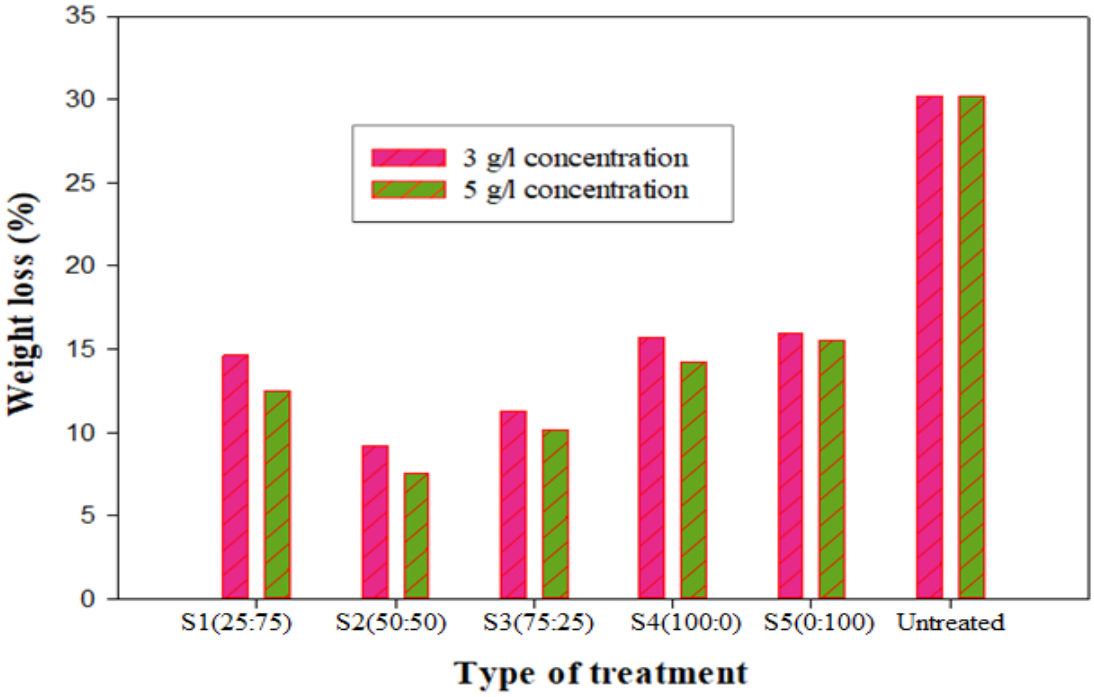


Figure 4.12: Weight loss after soil degradation test

According to table 4. 3F in appendix 4.3 and figure 4.13, the least degradation (7.54 %) occurred in the 5 g/l 50% solanum incanum fruit and 50% red onion peel extract-treated fabric (sample 2), and the second least degradation also occurred in treatment type (sample 2) with 3 g/l concentration. This proves the strong antibacterial activity of the combinatorial extract of solanum incanum fruit and red onion peel.

On the other hand, as compared, the weight loss of the fabric treated with pure red onion peel and solanum incanum fruit, weight loss was 15.988 %, for only red onion peel extract-treated fabric, and 15.727 %, for only solanum incanum fruit-treated fabric. Thus, solanum incanum fruit had the strongest antibacterial effect underground every test. According to Thilagavathi et al. (2005) who report on the development of eco-friendly antimicrobial textile finishes using herbs, soil degradation test of fabric proves both the antimicrobial and biodegradability nature of the fabric finished with herb extracts. Although the treated samples resist soil degradation moderately, soil degradation results prove that treated samples are still biodegradable and thus, environmentally friendly. The present finding was supported by Mondal et.al. (2019).

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The value of total phenol content in mg GAE/g DW was 2.657 ± 0.00078 for red onion peel and 1.415 ± 0.001 for solanum incanum fruit and the total flavonoid contents in mg QUE/g DW was 4.949 ± 0.0067 for solanum incanum fruit and 2.866 ± 0.0294 for red onion peel. It was observed that the fabric weight and bending length of the fabric increased after the application of herb and vegetable peel extract. The tensile strength, air permeability and wetting property of the fabric decreased after the application of extracts. There was a maximum percent reduction in the count of test bacterial after cotton fabric finished with 50:50 standard combinations of solanum incanum fruit and red onion peel with 5 g/l concentration. The results of laundering durability indicated that the fabric treated with 50:50 combinations of solanum incanum fruit and red onion peel extracts with 5 g/l concentrations retained their antibacterial activity 15 laundering washes, only 15.83% reduction was showed after 15 wash cycle. Thereafter a slight reduction in activity was observed. Even though the treated fabric resists soil degradation moderately, soil degradation results prove that treated samples are still biodegradable and thus, environmentally friend. In short, the treated fabric was antibacterial effective, biodegradable, and did not delay the comfort properties significantly.

5.2. Recommendation

In the present study, only total phenol and total flavonoid content were quantified. For further study, all the active constituent present in the peel of onion and fruit of solanum incanum can be quantified. Before the direct application of the treated fabric on human skin; the toxicity nature of the extract and the shelf life of the treated fabric can be studied because it may cause skin irritation. For the application of the extract against fungi, bacteria other than S. aureus and E. coli, insect repellent and antioxidant, the chemical composition of the extract not only the pure but also the combined extract should be analyzed. The cotton fabric treated with pure and high percentage red onion peel extract in combinatorial, showed a yellowish-brown colour. The combinatorial extracts of red onion peel and solanum incanum fruit is recommended use for multifunctional cotton fabric (antimicrobial and natural dye).

BIBLIOGRAPHY

Abebe, H., Gebre, T., & Haile, A. J. J. o. M. P. (2014). Phytochemical investigation on the roots of Solanum incanum, Hadiya Zone, Ethiopia. 2(2).

Ali, S. W., Purwar, R., Joshi, M., & Rajendran, S. J. C. (2014). Antibacterial properties of Aloe vera gel-finished cotton fabric. 21(3), 2063-2072.

Alkhalifah, D. H. M. J. I. J. o. S. E., & Science, A. (2016). Antimicrobial Activities of Solanum Incanum, Elettaria Cardamomum and Zingiber Officinale, used traditionally to treat pathogenic microbes. 2(3).

Ashokkumar, R., Ramaswamy, M. J. J. o. C. M., & Sciences, A. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. 3(1), 395-406.

Azwanida, N. J. M. A. P. (2015). A review on the extraction methods uses in medicinal plants, principle, strength, and limitation. 4(3), 3-8.

Chandrasekar, S., & Vijayakumar, S. G. (2017). APPLICATION OF CHITOSAN-HERBAL COMPOSITES FOR THE FABRICATION OF ANTIMICROBIAL COTTON FABRIC USED IN HEALTH CARE TEXTILES.

Cowan, M. M. J. C. m. r. (1999). Plant products as antimicrobial agents. 12(4), 564-582.

Dhiman, G., Chakraborty, J. J. F., & Textiles. (2015). Antimicrobial performance of cotton finished with triclosan, silver, and chitosan. 2(1), 13.

Doughari, J. H. (2012). Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In Phytochemicals-A global perspective of their role in nutrition and health: InTech.

El-Shafei, A., Shaarawy, S., Motawe, F., & Refaei, R. (2017). Herbal Extract as an Eco-friendly Antimicrobial Finishing of Cotton Fabric.

Eltaweel, M. (2013). Assessment of Antimicrobial Activity of Onion Extract (Allium cepa) on Staphylococcus aureus; in vitro study. Paper presented at the International Conference on Chemical, Agricultural and Medical Sciences.

Gao, Y., & Cranston, R. J. T. R. J. (2008). Recent advances in antimicrobial treatments of textiles. 78(1), 60-72.

Gopalakrishnan, M., Saravanan, D. J. F., & Europe, T. i. E. (2017). Antimicrobial Activity of Coleus ambonicus Herbal Finish on Cotton Fabric.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. J. P. r. (2002). Onions—a global benefit to health. 16(7), 603-615.

Gupta, V. (2016). UV protective and antibacterial finish on cotton using plant extracts. CCSHAU,

Harborne, A. (1998). Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media.

Indhumathi, T., & Mohandass, S. J. I. J. C. M. A. S. (2014). Efficacy of Ethanolic extract of Solanum incanum fruit extract for its antimicrobial activity. 3(6), 939-949.

Jepkoech, K. E., & Gakunga, N. J. (2016). Antimicrobial activity and phytochemical screening of Solanum incanum fruit extract against clinical samples of Staphylococcus aureus collecting from Nakuru Provincial General Hospital Laboratory, Kenya.

Kasiri, M. B., & Safapour, S. J. E. c. l. (2014). Natural dyes and antimicrobials for green treatment of textiles. 12(1), 1-13.

Kokate, C., Purohit, C., & Gokhale, S. J. P. (1996). Phytochemical tests. 35, 510-512.

Kumar, M. Y., Raghu, T., Kumar, P., Varghese, F., & Kotresh, T. (2017). Application of Enriched Fraction of Seabuckthorn Leaf Extract as Antimicrobial Finish on Technical Textile.

Lines, T., & Ono, M. J. P. (2006). FRS 1000, an extract of red onion peel, strongly inhibits phosphodiesterase 5A (PDE 5A). 13(4), 236-239.

Malpani, S. R. J. I. J. A. R. S. E. (2013). Antibacterial treatment on cotton fabric from neem oil, aloe vera, and tulsi. 2, 35-43.

Mohammad, F. J. A. S. C., & Engineering. (2015). Natural colorants in the presence of anchors so-called mordants as promising coloring and antimicrobial agents for textile materials. 3(10), 2361-2375.

Mohammed, M. A. S. A. (2006). ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS INDIGENOUS TO SUDAN. University of Khartoum,

Mondal, M. I. H., Saha, J. J. J. o. P., & Environment, t. (2019). Antimicrobial, UV Resistant and Thermal Comfort Properties of Chitosan-and Aloe vera-Modified Cotton Woven Fabric. 1-16.

Mortazavi, S. M., & Esmailzadeh, B. P. (2004). Application of mixtures of resin finishing to achieve some physical properties on interlining cotton fabrics: I-effect of stiffening and cross-linking agents.

Mujeeb, F., Bajpai, P., & Pathak, N. J. B. r. i. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. 2014.

Mukthy, A. A., Azim, A. Y. M. A. J. I. J. o. S. E., & Technology. (2014). Effects of resin finish on cotton blended woven fabrics. 3(7), 983-990.

Murugesh Babu, K., & Ravindra, K. J. T. J. o. T. T. I. (2015). Bioactive antimicrobial agents for finishing of textiles for health care products. 106(7), 706-717.

Nalankilli, G., & Tadesse, K. J. A. R. R. (2018). Antimicrobial cotton textiles by finishing with extracts of an Ethiopian plant (Solanum incanum) fruit. 12(2), 143-152.

Ortiz, M. J. M. F. P., & Technology. (2015). Antimicrobial activity of Onion and Ginger against two foodborne pathogens Escherichia Coli and Staphylococcus Aureus. 1(4), 1-9.

Palmer, G. (2016). Efficacy of silver treated textile for prevention of microbial activity. University of Otago,

Purwar, R. (2019). Antimicrobial textiles. The Impact and Prospects of Green Chemistry for Textile Technology (pp. 281-306): Elsevier.

Sahle, T., & Okbatinsae, G. (2017). Phytochemical investigation and antimicrobial activity of the fruit extract of Solanum incanum grown in Eritrea.

Sambo, H., Pam, C., & Dahiru, D. J. A., Bus. Tech. (2000). Effect of aqueous extract of Solanum incanum fruit on some serum biochemical parameters. 10, 82-86.

Şapcı, H., Yılmaz, F., Vural, C., Bahtiyari, M. İ., & Benli, H. J. I. J. o. S. M. (2017). Antimicrobial and Antifungal Activity of Fabrics Dyed with Viburnum opulus and Onion Skins. 4(3, Special Issue 1), 280-284.

Sathianarayanan, M., Bhat, N., Kokate, S., & Walunj, V. (2010). Antibacterial finish for cotton fabric from herbal products.

Silva, M., Barros, M., Almeida, R., Pilau, E., Pinto, E., Ferreira, A., . . . Santos, J. G. (2018). Multifunctionalization of cotton with onion skin extract. Paper presented at the IOP Conference Series: Materials Science and Engineering.

Simoncic, B., & Tomsic, B. J. T. R. J. (2010). Structures of novel antimicrobial agents for textiles-a review. 80(16), 1721-1737.

Singh, N., Punia, P., & Singh, V. J. I. J. C. M. A. S. (2017). Bacterial Resistance Finish on Cotton Fabric with Pomegranate and Onion Peel Extracts. 6(10), 1075-1079.

Singleton, V. L., Rossi, J. A. J. A. j. o. E., & Viticulture. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. 16(3), 144-158.

Škerget, M., Majhenič, L., Bezjak, M., Knez, Ž. J. C., & quarterly, b. e. (2009). Antioxidant, radical scavenging and antimicrobial activities of red onion (Allium cepa L) skin and edible part extracts. 23(4), 435-444.

Sood, A. (2014). Application of Herbal Extracts on Covering Fabric of Sanitary Napkins for Bacterial Resistance. CCSHAU,

Sunder, A. E., & Nalankilli, G. (2012). Polyfunctional finishes on cotton textiles.

Tadesse, K., & Nalankilli, G. (2017). Antimicrobial Finishing of Cotton with Extracts of Solanum Incanum Fruit.

Tawiah, B., Badoe, W., Fu, S. J. F., & Europe, T. i. E. (2016). Advances in the development of antimicrobial agents for textiles: The quest for natural products. Review.

Thilagavathi, G., Rajendrakumar, K., & Rajendran, R. (2005). Development of ecofriendly antimicrobial textile finishes using herbs.

Thirumurugan, K. J. s. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. 1, 7.

Tsuji, W. (1971). On the Lowering of the Tensile Strength of Cotton Fiber Caused by the Intermolecular Crosslinking (Special Issue on Polymer Chemistry, VIII).

Unango, F. J., Ramasamy, K. M. J. I. R. J. o. S., & Technology. (2019). A review on the investigation of biologically active natural compounds on cotton fabrics as an antibacterial textile finishing. 1(1), 49-55.

Varier, K. M., Milton, M., Arulvasu, C., Gajendran, B. J. J. o. A., & Research, I. (2013). Evaluation of antibacterial properties of selected red seaweeds from Rameshwaram, Tamil Nadu, India. 1(11), 667-670.

Viera, V., Piovesan, N., Rodrigues, J., de O Mello, R., Prestes, R., dos Santos, R., . . . Kubota, E. J. I. F. R. J. (2017). Extraction of phenolic compounds and evaluation of the antioxidant and antimicrobial capacity of red onion skin (Allium cepa L.). 24(3).

Waweru, C. M., Muthamia, J. M., & Otaye, D. O. J. A. i. A. (2017). Potential of Sodom Apple (Solanum incanum L.) Fruit Extracts in the Management of Chilli Root Knot Disease in Nakuru County, Kenya. 2017.

Windler, L., Height, M., & Nowack, B. J. E. i. (2013). Comparative evaluation of antimicrobials for textile applications. 53, 62-73.

APPENDIX

Appendix 3.1: List of Plant Exhibiting Antibacterial Properties

Scientific name	Common name	Parts	Reference
Zingiber officinale	Ginger	Rizome	(Ortiz & Technology, 2015)
Punica granatum	pomegranate	Rings	(Singh et al., 2017)
Aegle marmelos (l)	Bael	Leaves	(Mujeeb et al., 2014)
Aloe barbadensis Miller	Aloe Vera	(gel) leaves	(Ali et al., 2014)
Portulaca oleracea	Purslane	Stem and leaves	(Gupta, 2016)
Syzygium cumini (L.)	Black plum	Leaves	(Gupta, 2016)
Psidium guajava (L.)	Guava	Leaves	(Gupta, 2016)
Azadirachta indica	Neem	Leaves and fruit	(Malpani, 2013)
Osmium basilcum	Tulsi	Leaves	(Malpani, 2013)

Appendix 3.2: Reagent preparation for phytochemical analysis:

1 % ammonia: 1 ml of ammonia was dissolved in 99 ml of distilled water.

1 % aluminum chloride: 1 g of aluminum chloride was dissolved in 100 ml of distilled water.

Wagner's reagent: it is used for alkaloids detection.

Solution (a) 2 g of iodine was dissolved in 60 ml of distilled water.

(b) 6 g of potassium iodide was dissolved in 20 ml of distilled water. Solution (a) and (b) were mixed and the volume was adjusted to 100 ml with distilled water.

Preparation of reagents:

Preparation of 10% aluminum chloride: 10 grams of AlCl3 was taken in 100 ml of volumetric flask and a small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding the required amount of distilled water.

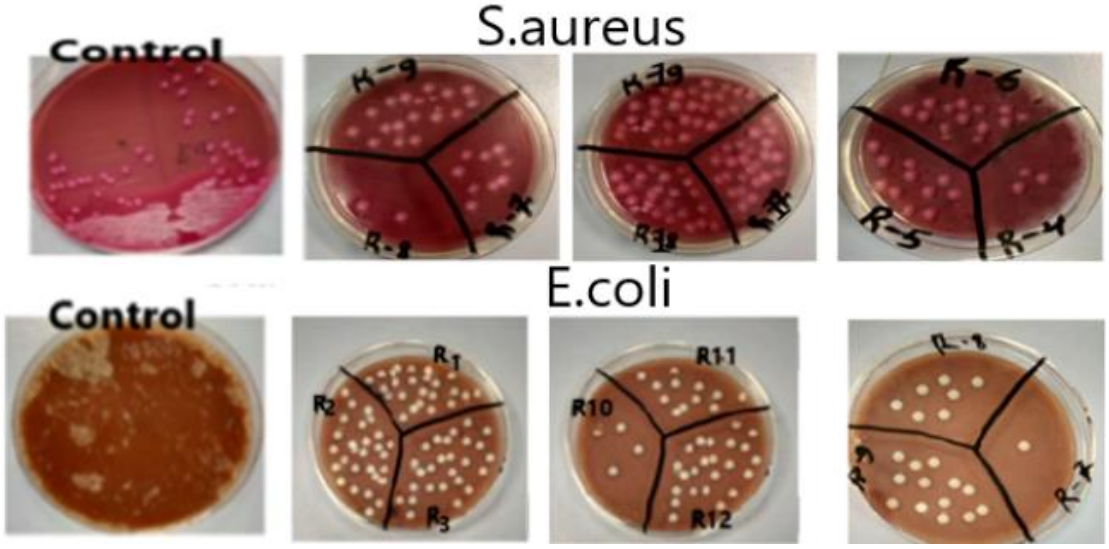
Preparation of 1 M Sodom hydroxide solution: 4 grams of NaOH was taken in 100 ml of volumetric flask and a small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding the required amount of distilled water.

Preparation of standard quercetin acid solution: 0.2 grams of quercetin was dissolved into 200ml distilled water, so the concentration of the solution is 0.001 gram/ml or 1000µg/ml. This is called the stock solution. Then serial dilution was performed in order to prepare differently concentrated of solution (0 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml) which is used for preparing a calibration curve.

Preparation of 5% Sodium Nitrite: 5 grams of NaNO2 was taken in 100 ml of volumetric flask and a small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding the required amount of distilled water.

Preparation of Blank: Blank consists of all the reagents, except for the extract or quercetin/ gallic acid standard solution was substituted with 1ml of distilled water.

Appendix 4.1: Figures About Antibacterial activity of cotton fabric by AATCC100-2004 method against S. aureus and E. coli



Where R-7, R-8, and R-9 =Fabric treated with 50:50, 75:25 and 25:75 combinations of solanum incanum fruit and onion peel at 5 g/l concentration respectively.

R10, R11, and R12= Fabric treated with 50:50, 75:25 and 25:75 combinations of solanum incanum fruit and onion peel at 3g/l concentration respectively.

R1 and R2 =Fabric treated with pure solanum incanum fruit and onion peel extract at 5 g/l concentration respectively.

R-4 and R-5= Fabric treated with 75:25 and 25:75 combinations of solanum incanum fruit and onion peel at 5g/l concentration respectively.

R-18 and R-19 =Fabric treated with pure solanum incanum fruit and onion peel extract at 3 g/l concentration respectively.

R6 and R-17= Fabric treated with pure solanum incanum fruit and onion peel extract at 5 g/l concentration respectively.

R3= Fabric treated with pure solanum incanum fruit extract at 3 g/l concentration.

R-7, R-8, and R-9 = Fabric treated with 75:25, 50:50 and 25: 75 combinations of solanum incanum fruit and red onion peel extract at 3 g/l concentration respectively.

Appendix 4.2: Calibration curve for total phenol and flavonoid determinations

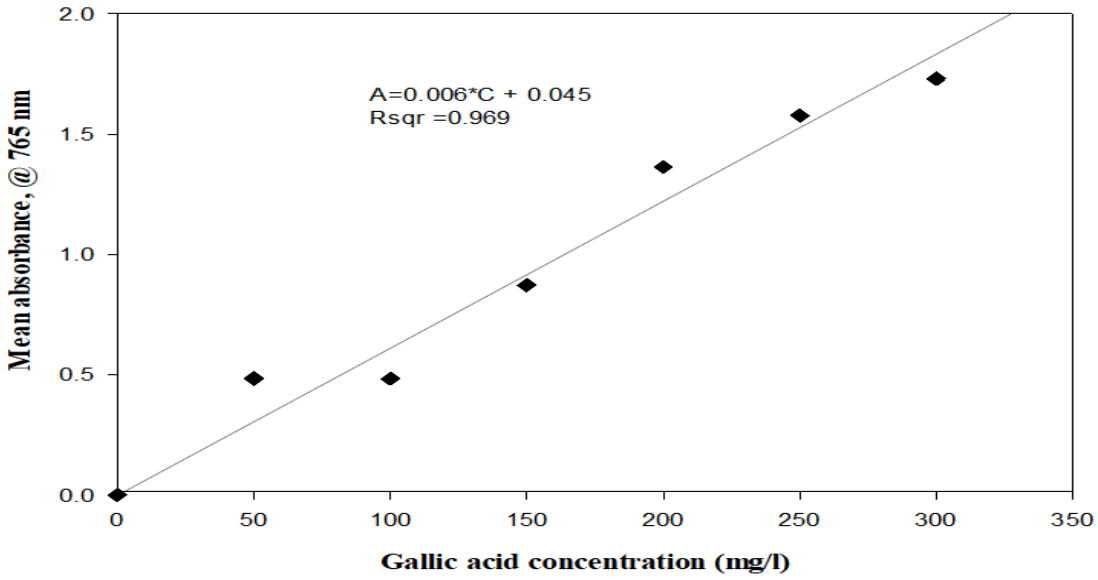


Figure 4.2A: Calibration curve for standard Gallic acid

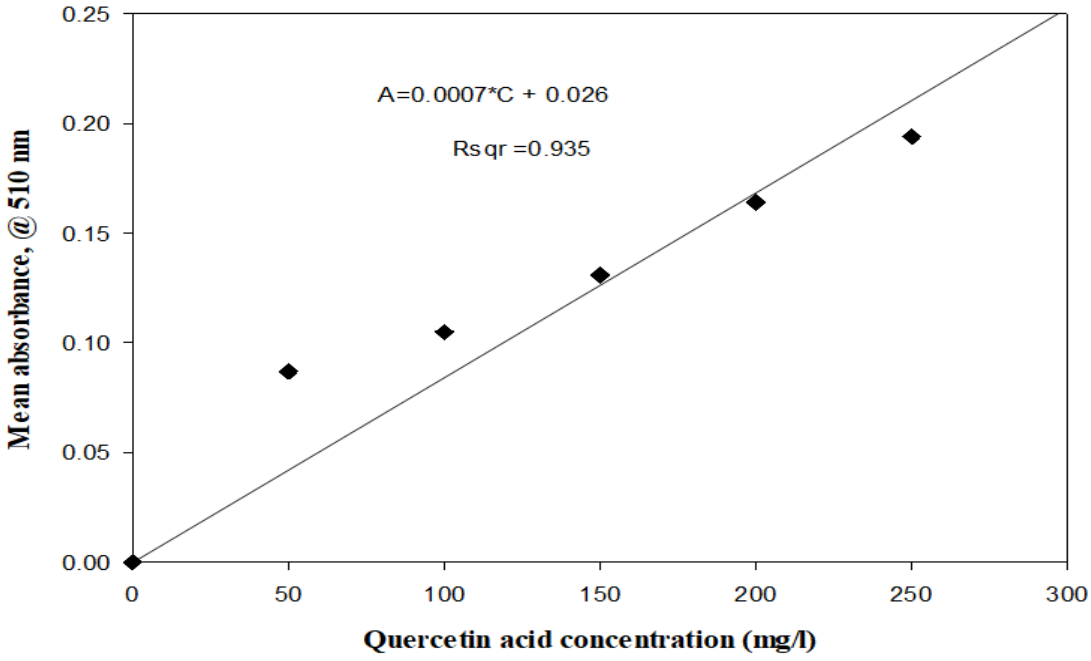


Figure 4.2B: Calibration curve for standard quercetin acid

Appendix 4.3: Tables obtained from the experimental result

Table 4.3A: Effect of combinatorial extracts on bending length of fabric

standard combination (S: R)	Bending length @ concentration 3g/l (cm)	Bending length @ concentration 5 g/l (cm)
S1 (25:75)	2.05	2.09
S2 (50:50)	2.02	2.04
S3 (75:25)	1.995	2.025
S4 (100:0)	1.985	2.0
S5 (0:100)	2.07	2.10
Untreated	2	2

Table 4.3B: Effect of combinatorial extract ratio on weight add on a percentage

Concentration (g/l)	Weight adds on a percentage (%)				
	The red onion peel extract	Solanum incanum fruit extract	Combination (solanum incanum fruit: red onion peel) extract		
			25:75	50:50	75:25
3	3.45	2.07	8.20	7.19	6.13
5	5.2	3.38	10.32	9.44	8.52

Table 4.3C: Effect of combinatorial extracts on the air permeability of the fabric

Concentration (g/l)	Air permeability (cm3/cm2.s)				
	Pure Red onion peel extract (Mean ± S. E)	Pure solanum incanum fruit extract (Mean ± S. E)	Combination (solanum incanum fruit: red onion peel) extract		
			25:75(Mean ± S. E)	50:50(Mean ± S. E)	75:25 (Mean ± S. E)
3	39.775 ±1.69	43.425 ± 1.106	38.75 ± 1.17	40.257± 0.95	42.025 ± 0.936
5	39.325 ±1.56	41.6 ±1.25	38.275 ±0.844	37.85 ± 1.45	41.05 ± 0.78
Untreated	46.675 ±1.14				

Table 4.3D: Effect of combinatorial extracts on water absorbency of the fabric

Treatment type	Water absorbency (sec.) @ conc.3gpl (Mean ± S.E)	Water absorbency (sec.) @ conc.5gpl (Mean ± S.E)
S1(25:75)	8.25 ± 0.433	12.25 ± 1.299
S2(50:50)	7.25 ± 0.433	10.5 ± 0.866
S3(75:25)	5.5 ± 0.5	9.25 ±0.433
S4(100:0)	4.25 ± 0.433	8.25 ± 2.51
S5(0:100)	15.25 ± 0.433	18 ± 0.000
Untreated	1.25 ± 0.433	1.25 ± 0.433

S.E- Standard error

Table 4.3E: Effect of combinatorial extracts on tensile strength of the fabric

Type of treatment	Tensile strength (N)@ conc. 3 g/l				Elongation (%)		Tensile strength (N)@ conc. 5g/l				Elongation (%)	
	Warp	% loss	Weft	%loss	Warp	Weft	Warp	% loss	Weft	% loss	Warp	Weft
Untreated	125	-	132	-	9.348	14.335	125	-	132	-	9.348	14.335
S1 (25:75)	106	15.2	114	13.63	14.58	7.834	72	42.4	101	23.48	12.730	7.314
S2 (50:50)	109	12.8	115	12.87	15.01	7.525	80	36	111	15.90	7.525	15.013
S3 (75:25)	105	16	117	11.36	12.10	7.156	89	28.8	110	16.67	13.291	7.811
S4(100:0)	110	12	119	9.85	13.91	9.266	100	20	114	13.63	14.48	9.055
S5(0:100)	100	20	112	15.15	14.48	9.055	70	44	100	24.24	9.348	14.335

Table 4.3F: Weight loss after soil degradation test of the fabric

Type of treatment	Weight loss after soil degradation test (%) @ 3 g/l conc.	Weight loss after soil degradation test (%) @ 5g/l conc.
S1	14.603 ± 0.048	12.471 ± 0.300
S2	9.197 ± 0.950	7.54 ± 0.100
S3	11.248 ± 0.180	10.125 ± 0.394
S4	15.727 ± 0.180	14.225 ± 0.410
S5	15.988 ± 0.409	15.551 ± 0.359
Untreated	30.227 ± 0.181	30.227 ± 0.181

Table 4.3G: Wash durability of the treated fabric against E. coli and S. aureus bacteria

Type of treatment	Test bacteria	Bacterial reduction	Wash cycle			
			0	5	10	15
Solanum incanum fruit	E.coli	%	98.00	90.00	87.50	57.50
	S.aureus	%	98.83	92.42	88.33	70.00
Red onion peel	E.coli	%	97.67	89.17	85.00	49.17
	S.aureus	%	98.00	91.67	85.83	55.83
50:50 combination	E.coli	%	99.92	97.58	92.50	84.17
	S.aureus	%	100	98.58	93.33	85.00
Silver nitrate	E.coli	%	100	98.25	85	60.00
	S.aureus	%	100	98.5	86.67	65.00

Table 4.3 H: Antibacterial activity of cotton fabric treated by solanum incanum fruit and red onion peel against S. aureus and E. coli

Conc.g/l	Bacteria	Bacterial reduction	Type of treatment				
			Solanum incanum fruit	Red onion peel	Combination of solanum incanum to onion peel extract		
					50:50	25:75	75:25
3	E.coli	%	97.83	97.17	99.63	98.08	99.00
	S.aureus	%	97.92	97.50	99.83	98.42	99.25
5	E.coli	%	98.00	97.67	99.92	98.92	99.42
	S.aureus	%	98.83	98.00	100	99.08	99.75
Untreated	Confluent growth						