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Antimicrobial Cotton Textiles by Finishing with Extracts of an Ethiopian plant (*Solanum Incanum)* Fruit

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Abstract

The use of natural fruits that are non-toxic, non-allergic and eco-friendly on textile as antimicrobial has become a matter of significant importance, may be, as a result of increased environmental awareness and because they prevent some hazardous synthetic antimicrobial. Antimicrobial finishing treatment on cotton textile fabric was carried out using extracts from natural plant *Solanum Incanum* fruit. The active substance was extracted from fruit by using Soxhlet apparatus and applied on the fabric in different concentrations viz. 15, 20, 25 and 30g/l. The antimicrobial activity of the treated fabric was assessed by Disc Diffusion (SN 195 920) method. High zone of inhibition obtained from 30g/l concentration. The durability of the finish product after five wash for zone of inhibition of fabric was also studied and found to be good. The aim of the present work is imparting antimicrobial finish on cotton by using natural fruit extract to fabric and to reduce the effect of microorganism on human body and a fabric.

Key words: Antimicrobial, S*olanum Incanum*, Disc Diffusion, zone of inhibition, durability of finish

Introduction

Antimicrobials control destroys or suppresses the growth of microorganisms and their negative effects of odour, staining and deterioration. Antimicrobial finishing prevents or inhibits the growth of microorganisms or microbes. The vast majority of antimicrobials work by leaching or moving from

the surface on which they are applied. Besides affecting durability and useful life, leaching technologies have the potential to cause a variety of other problems when used in garments. These include their negative effects because they can contact the skin and potentially affect the normal skin bacteria, cross the skin barrier, and/or have the potential to cause rashes and other skin irritations. When applied, the technology actually polymerizes with the substrate making the surface antimicrobial. This type of antimicrobial technology is used in textiles that are likely to have human contact or where durability is of value [Schulz et al, 2013; Reza Ghorbani and Carlo Leifert, 2005].

Natural antimicrobials derived from plants have been recognized for centuries, but only scientifically confirmed in the last 30 years. The antimicrobial efficacy of components in plants depends on the chemical structure of active components and their concentration. There are various chemical components present in plants with antimicrobial effect including saponin, triterpenoids flavonoids, thiosulfinates, glucosinolates, phenolics, and organic acids. However, the main components in plants with antimicrobial activity are phenolic compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, acids, and is flavonoids [Sambo et al, 2012; Haruna Sarah Sambu et al, 2012; Sell, 2013, Ovesna et al, 2004; Cseke et al, 2006; Pan et al, 2011; Brown, 1980; Cowan, 1999] . For example, the antibacterial activity of 46 extracts from spices and herbs was suggested to be associated with the presence of phenolic constituents. The researchers have reported that all the tested spices have a strong antibacterial effect against *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Salmonella anatum. Solanum Incanum* is also one of the plants that are used for antimicrobial [Saeed et al, 2013].

Solanum Incanum is one of about 1,500 *Solanum* species in the world. Widely distributed in the Horn of Africa it shows characteristic thorny leaves, yellow fruits and blue flowers with yellow pistils. Throughout tropical Africa ashore throat, angina, stomach pain, colic, headache, painful menstruation and liver pain are treated with *Solanum Incanum*. In addition, the fruit of *Solanum Incanum* is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania [Sambo et al, 2012; Haruna Sarah Sambu et al, 2012]

A number of secondary metabolites have been noted for their antimicrobial activity. Secondary metabolites with antimicrobial activity can be found in most organisms including plants such as fruits, vegetables, seeds, herb, and spices, animal sources such as milk, eggs, and tissues [Saeed et al, 2013]. Plants such as *Menthapiperita, Rosmarinus officinalis, Arrabidaeachica, Tabebuiaavellanedae, Punicagranatum and Syzygiumcumini* have been used due to their antimicrobial properties. Recent studies strongly support that contamination of textiles in clinical settings may contribute to the dispersal of pathogens to the air which then settle down and infect the immediate and non-immediate environment [Isabel and Gouveia, 2010]. It becomes very important to finish all garments where the chance of bacterial growth is high and the safety is paramount. This may include medical garments, sanitary napkins, socks, underwear, disposable wipes, carpets etc. [Patel and Tandel, 2005].

Applications of natural antimicrobial agents have 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 [Muhammad Furaqan Khurshid et al, 2015] Natural finishes have many advantages such as nontoxic, nonirritant, biodegradable, cost effective, easy availability, etc. Concern for the green environment along with public awareness led to the innovation of many new natural finishes [Sasikala et al, 2016]. The relatively lower incidence of adverse reactions of herbal products as compared to modern synthetic pharmaceuticals, coupled with their reduced cost, can be exploited as an attractive eco-friendly alternative to synthetic antimicrobial agents for textile application [Chandrasekaran et al, 2012].

Antibacterial test methods generally used are agar diffusion test and parallel streak method. The zone of inhibition of both *E.coli and S.aureus* are determined accurately [Haruna Sarah Sambu et al. 2012].

After that checking, antibacterial effect by quantitative test and qualitative test is possible [Sasikala et al, 2016, Jothi, 2009, Mahesh et al, 2011].

The presence of chemicals in fruit is known to have antibacterial, antifungal, antioxidant, anticancer, anti- inflammatory and hypoglycaemic activity [Sambo et al, 2012]. The plant extracts showed varying degree of antibacterial activity against the test organisms. Large zone of inhibition were seen on the plate with *S.pyogenes and S.aureus* [Owino et al, 2013]. The clear zone of growth inhibition was noted around the disc due to diffusion of drug and growth of bacteria when *Solanum Incanum* fruit extract was used. [Indhumathi and Mohandass, 2014]. The methanolic extract of *solanum incanum* has better activity on *P.aeruginos, S.aureus and B.subtilis* than other [Beaman-Mbaya and Muhammed, 1976]. The crystals of this compound were effective inhibitors of the growth of gram-positive and negative bacteria, yeasts, dermatophytes, and some pathogens of agricultural produce. High concentrations of the substance caused hemolysis of erythrocytes (Dalal Hussien and Alkhalifah, 2016).

The aim of the present work is imparting antimicrobial finish on cotton by using natural fruit extract to fabric and to reduce the effect of microorganism on human body and a fabric.

Materials and Methods

Materials

Fabric: A commercially prepared and dyed 100% cotton fabric of 24 ends /inch and 18 picks/inch were used.

Antimicrobial source: *Solanum Incanum* fruit (Amharic name Enbuye). It was collected from Ankober Amhara region and Arsi and Oromia region of Ethiopia.

Chemicals: Mueller-Hinton agar was used as media of growing of bacteria and potato dextrose agar was also used as media for growing of fungi. Sulfuric acid (H_2SO_4) and Barium chloride ($BaCl_2.2H_2O$) were used for McFarland standard preparation. Standard soap 1.25 g/l for washing to check antimicrobial effect after washing..

Equipment: The various equipments used are Soxhlet apparatus for extracting the active ingredient from powder with water, Launder-O-meter to assess the durability of finish, Incubator for growing of microorganism for 24hr, Autoclave for serialization different equipment and media, Chamber for preparation of everything that used antimicrobial treatment like media preparation, McFarland standard preparation was to put treated fabric on petri dish and Petri dish for putting media in growing of microorganism. L shape glass rod was used for uniformly distribution of a bacteria in petri dish; Loop during dilution was to transfer same number of bacteria from one test tube to another; Conical flux for preparation of different media, Test tube for putting maximum recovery that is used for dilution, Padding mangle for applying antimicrobial on to fabric, Dryer for removing the excess water from fabric after applying finishing agent to remove, ZM 100 ULTRA Centrifugal mill for reducing the size of fruit into powder form, Caliper for measuring the zone of inhibition of bacterial, Universal strength tester for measuring of the tensile strength and elongation of break antimicrobial treated fabric.

Methods

Extraction from Plant *Solanum Incanum Fruit*

Collected *solanum Incanum* fruit from available area was washed with water to remove dust and other impurity, and then dried under sun light for 3 days by cutting it into small pieces. Dried fruit were subjected for size reduction to powder by using grinder (ZM 100 ULTRA Centrifugal mill). The

dried powder, 150 g was exhaustively extracted with water using Soxhlet apparatus for seven days. The extract was later concentrated to dryness on stove and weighed. The extract was kept in a tight container in refrigerator.

Antimicrobial Finishes of Textile

The fabrics were immersed in the 15, 20, 25 and 30gpl concentration of extracted *solanum Incanum* for fifteen min and padded on padding mangle individually in the presence of acetic acid to maintain 4.5pH to get a wet pick up of 80% on weight of the fabric. The fabric was then dried at 80^0C for 3min and cured at 160^0C for 3min.

Antimicrobial Activity Assessment

Antimicrobial activity was evaluated by qualitative test methods. The method is known as Disc Diffusion or disc Agar (SN 195 920) and Parallel Streak (AATCC 147). Procedure used for preparing the above two methods are the same. The main difference between Parallel Streak and Disk Diffusion is bacteria inoculating. Disk Diffusion or Ager Diffusion method of bacteria was inoculating throughout the volume of petri dish. But in Parallel Strike method, bacteria was not suspended all volume of a media. Loop full of the diluted inoculums suspension in five consecutive streaks. Five parallel streaks in varying concentration was prepared with samples, which have been cut to be rectangular in shape and measuring 25 x 50 mm, as recommended by the method, and are evenly placed across the five Parallel Streaks.

Media Preparation

Types of general media that were used for growing bacteria and fungi or mold are Mueller Hinton agar and Potato Dextrose agar. Mueller Hinton agar was used for all type of bacteria growing 38.9 gram in 1000 ml. and depending on number of Petri dish calculation of concentration was done. One Petri dish contains 40-50 ml but for antimicrobial treatment media fill was half of Petri dish. Potato Dextrose agar, another type of media was also used for growth of mold 39.5 gram in 1000ml. It was also prepared depending on number of Petri dish and media fill. It was dissolved in distilled water and put on stove for uniform dilution for few seconds. After the dilution completed, all media were put in Petri dish, test tube and other material in autoclave for sterilization for 15min with 120^oC, 20 bar pressure. Sterilization was completed, then equipment was put in chamber to reduce contamination. A bacterium inoculating was suspended on Petri dish uniformly by using swabor L shape glass road. To reduce the suspension of bacteria, in one plate standard known as McFarland were used as a reference to adjust the turbidity of bacterial suspension.

McFarland Standard Preparation

i. Add 0.05mlof 1.175%w/v $BaCl_2.2H_2O$ to 9.95ml of 1% v/v $H_2SO_4$with constant stirring to maintain a suspension in glass tube.

ii. Add bacteria colonies to another tube by using inoculating loop or needle up to find the same turbidity with standard. The similarity of the two tube measured by putting both in front of a wickerham card.

McFarland standard is equivalent to a bacterial suspension containing between 1 x 108 and 2 x 108 CFU (colony forming unit)/ml. based on the above number of bacteria that found in one petri dish to much high so, to reduce the amount of bacteria by using maximum recovery diluted bacteria and reduced number into 1*105 CFU/ml. The CFU/ml can be calculated using the formula: CFU/ml = (no. of colonies x dilution factor) / volume of culture plate

The first test tube contain 9ml of maximum recovery and the amount of bacteria $1*10^8$ CFU /ml from this test tube inject1ml solution into second test tube by using syringe. The second test tube also

contains 9ml maximum recovery and the amount of bacteria contains $1*10^7$ CFU /ml. From the second test tube inject 1ml solution into third test tube and the amount of bacteria that found in third test tube is $1*10^6$ CFU /ml. From the third test tube also contain 9ml maximum recovery and inject1ml solution into and forth test tube and the amount of bacteria that found in the fourth test tube $1*10^5$ CFU /ml. From the fourth test tube using sterile swab dip into inoculum tube than rim the plate with the swab to pick up excess liquid. Finally press the treated fabric gently on media and put in incubator of 18-24 hr. than directly by using ruler or caliper measure zone of inhibition.

Finish Durability to Washing

The finished samples were washed using 1.25g/l standard detergent for 15 minutes at 40° C. After 5 washes the antimicrobial effect was assessed using the above mentioned procedure.

Tensile Strength Test after Antimicrobial Treatment

Universal strength tester machine was used for measuring tensile strength and breaking elongation of treated sample. It measured in both warp and weft direction. A sample amount that used for testing width 5cm and length 10cm for both warp and weft direction. The standard that used to find result was ISO 13934 part 1. Average of 5 samples is reported.

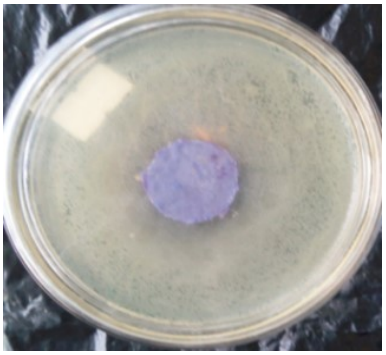
Results and Discussion

Antimicrobial Activity of *Solanum Incanum* Treated Sample (Agar Diffusion Test and Parallel Streaks)

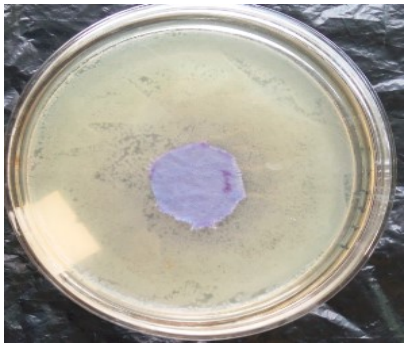
Figures1-4 show the result of disc Diffusion Test for antimicrobial effectiveness against *E.coli and S.aureus.* The zone of bacterial inhibition is indicated by *SolanumIncanum* concentration 15g/l, 20g/l, 25g/l and 30g/l around the specimen is good. But, depending on the concentration of solution zone of inhibition is different. Fig-1 shows the result of disc diffusion test for antimicrobial effectiveness against *E.coli and S. aureus.* The zone of bacterial inhibition is indicated by *Solanum Incanum* concentration 15g/l around the specimen was good but the zone of inhibition was less than the other three. It was because of low concentration so, release of active substance from the fabric surface also small. Fig-2 shows the result of disc diffusion test for antimicrobial effectiveness against *E.coli and S. aureus*.

The zone of bacterial inhibition was indicated by *Solanum Incanum* concentration 20g/l around the specimen was greater than concentration 15g/l but it result less whenrelated to concentration 25g/l and 30g/l. It was because of concentration of *Solanum Incanum* and it also released less amount of active substance than 25g/l and 30g/l concentration. Fig-3 shows the result of disc diffusion test for antimicrobial effectiveness against *E.coli and S. aureus*. The zone of bacterial inhibition was indicated by *Solanum Incanum* concentration 25g/l around the specimen was greater than concentration 15g/l and 20g/l but, its zone of inhibition less than concentration 30g/. Because of the amount of concentration difference and the active substance that released from *Solanum Incanum* fruit. Fig-4 shows the result of disc diffusion test for antimicrobial effectiveness against *E.coli and S. aureus.*

The zone of bacterial inhibition was indicated by *Solanum Incanum* concentration 30g/l around the specimen is higher than other concentration like concentration 15g/l, 20g/l and 25g/l. because it has high concentration and hence, the active substance that released from *Solanum Incanum* fruit was high.



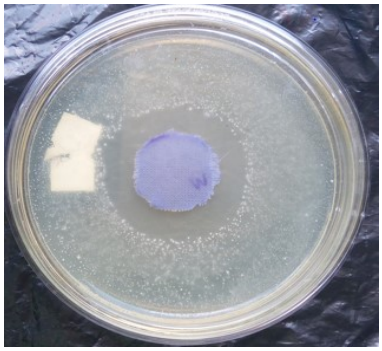
S. aureus



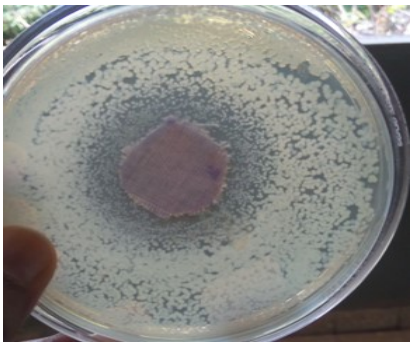
E.coli

Fig-1: Untreated Sample by Disc Diffusion Method for both *E. coli and S. aureus*.

The figure 1 show for both *S. aureus and E.coli* there was no clear zone around the fabric or no zone of inhibition the bacteria growth up to fabric end.



S. aureus



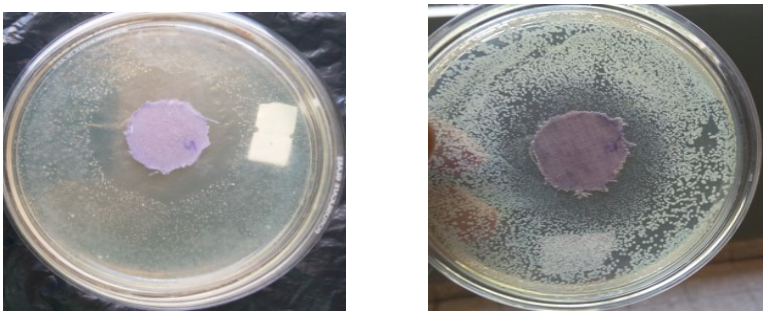
E.coli

Fig-2: Antimicrobial Activity of *Solanum Incanum* Fruit Treated Sample with Concentration of 15g/l Disc diffusion Zone of Inhibition against *E.coli and S. aureus.*

On 15g/l concentration, Fig-1 shows clear zone round the fabric which indicate, the material that was used as antimicrobial having antimicrobial effect. Before putting of fabric on the media both S. aureus and E.coli bacterias, they were are distributed throughout the volume of petri dish but, after putting 100mm treated fabric on bacteria contain media the active substance that released from treated fabric inhibit the growth the bacteria. Zone of inhibition of 17g/l concentration for *S.aureus* 55mm or 5.5cm. Zone of inhibition of 15g/l concentration for *E.coli* 45mm or 4.5cm.

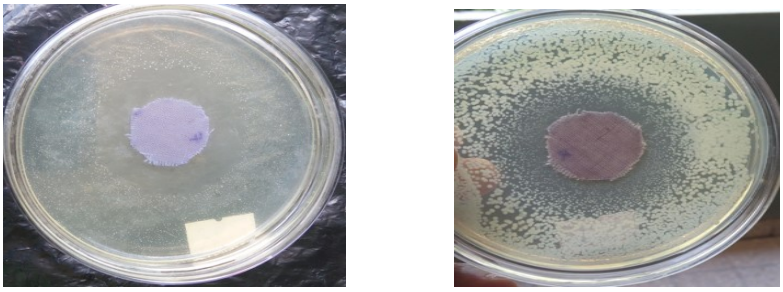
Zone of inhibition of 20g/l concentration for *S. aureus* was 75mm or 7.5cm and for *E.coli* 52mm or 5.2cm. Zone of inhibition of 25g/l concentration for *S. aureus was* 78mm or 7.8cm and for *E.coli* 60mm or 6cm. Zone of inhibition that was measured with 30g/l concentration for *S. aureus* was 80mm or 8cmand for *E.coli* was 70mm or 7cm (Fig.3,4& 5).

The Table-1 shows zone of inhibition of treated fabric in different concentration of *Solanum Incanum* antimicrobial agent by numeric description.



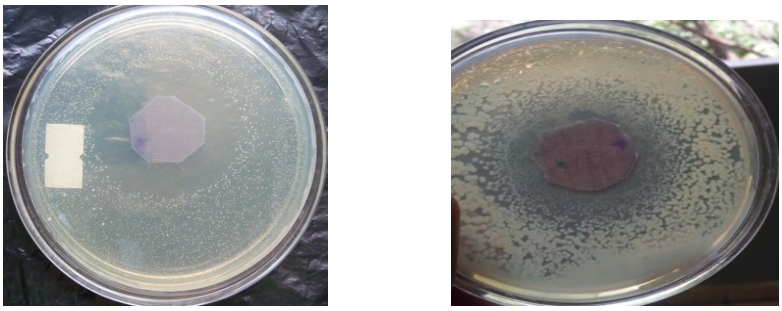
S. aureus. *E.coli.*

Fig-3: Antimicrobial Activity of *Solanum Incanum* Fruit Treated Sample with Concentration of 20g/l Disc Diffusion Zone of Inhibition Against *E. coli and S. aureus.*



S. aureus *E.coli*

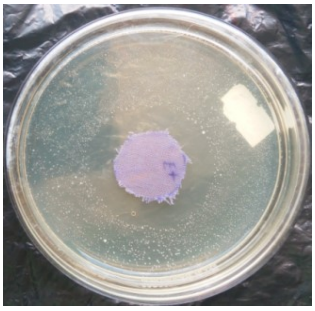
Fig-4: Antimicrobial Activity of *Solanum Incanum* Fruit Treated Sample with Concentration of 25g/l Disc Diffusion Zone of Inhibition against *E. coli and S. aureus*.

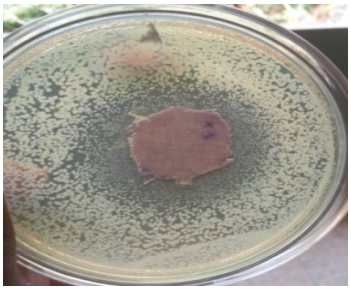


S. aureus *E.coli*

Fig-5: Antimicrobial Activity of *Solanum Incanum* Fruit Treated Sample with Concentration of 30g/l Disc Diffusion Zone of Inhibition against *E.coli and S. aureus.*

Based on the above results, first choice concentration was 30g/l because it has great or high zone of bacterial inhibition when compared with concentration less than 30g/l. Also it has been observed that with increasing concentration, the antimicrobial effect also increased. Though the length (millimeter) increase in antimicrobial effect is significant when we increase from 25gpl to 30gpl in case of *E.coli,* the corresponding increase in *S. aureus* is only marginal (2mm) and hence, it was decided to use 30gpl. So, the durability of finishing was tested only with 30g/l concentration of *Solanum Incanum* antimicrobial agent treated sample.





E.coli *S. aureus*

***Fig-6** : Antimicrobial Activity of* Solanum Incanum *Fruit Treated Sample with Concentration of 30g/l Disc Diffusion Zone of Inhibition Against* E.coli and S. aureus *After 5 Washing*.

After five washes, treated sample zone of inhibition is decreased by some amount. For *S. aureus* zone of inhibition of treated fabric with *Solanum Incanum*fruit antimicrobial agent is 65mm from 80mm and for *E.coli* zone of inhibition of treated fabric it was45mm from 70mm. However, the decrease is only 15 mm (18.75%) which indicates that significant antimicrobial activity was still present in the finished fabric. With proper use of a binder or cross-linking agents, it is expected that the permanency of the finish could be improved.

Effect of Finishing Treatment on Physical Properties

The result of tensile strength and elongation of the untreated and treated fabric is given in table-2. From the table, it is clear that there was loss in strength and increasing elongation at break.

With the increasing concentration of finishing agent, the strength loss was also increasing. However, the loss in strength was not significant that it was about 6.7 % even for the fabric finished with 30g/l finishing agent.

Conclusion

Based on the experimental results, it was concluded that high antimicrobial effect on cotton fabric can be achieved with concentration of 30g/l *Solanum Incanum* fruit extract. It is expected to be very useful, if applied on textiles such as innerwear and fabrics that is used as hygiene fabric, hospital bedsheet and so on. The durability of the finish is also found to be good that even after 5 washes, only 18.75% of antimicrobial activity was lost. The physical properties such as tensile strength and elongation were not affected much due to these finishing treatments.

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Table- 1: Antimicrobial Effect of Treated Sample

Concentration	Zone in mm	
	S. aureus	*E.coli*
Untreated	0	0
15g/l	55	45
20g/l	75	52
25g/l	78	60
30g/l	80	70

Table -2: The Effect of Finishing Treatment on Physical Properties.

Concentration	Tensile strength (N)				Elongation (%)	
	warp	% loss	weft	% loss	warp	weft
Untreated	284	-	281	-	8.57	13.00
15g/l	284	0	279	0.70	9.52	14.49
20g/l	274	3.52	266	5.33	10.80	15.29
25g/l	269	5.28	258	8.10	11.06	16.56
30g/l	265	6.69	254	9.60	12.07	17.86