HYGENIC ADULT/PATIENT DIAPER DEVELOPMENT AND PRODUCTION

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HYGENIC ADULT/PATIENT DIAPER PRODUCTION AND DEVELOPMENT

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ABSTRACT

HYGENIC ADULT/PATIENT DIAPER DEVELOPMENT AND PRODUCTION

Microorganisms can easily reproduce in humid environments containing nutrients and cause diseases in humans. For this reason, diapers used by patients or adults, who are unable to control fecal or urinary excretory, create a suitable growing medium for opportunistic pathogens.

In the current study, it is aimed to design and develop patient or adult diapers which have antimicrobial properties using boron compounds and Pluronic polymers (Poloxamer). For this purpose, Diaper dermatitis related pathogenic microorganisms (bacteria, yeast and fungi) were used to conduct antimicrobial activity tests. Boron, Pluronic polymers (F68 and F127), prepared at different doses, and their combinations were tested for their antimicrobial activities against a broad range of microorganisms. As a result of in-vitro tests, diapers containing boron and Pluronic combination were found to display remarkable antimicrobial properties.

The use of adult or patient diapers is expected to grow due to increasing population of old people in the future. Therefore, the objective of this project was to develop disposable adult and/or patient diapers providing higher comfort compared to commercially available products, prevent problems related to diaper usage, have high leakage barrier and posses antimicrobial properties. This study will create a competition within national and international arena and access to market with the antimicrobial adult and/or patient diaper developed as a consequence of this study.

ÖZET

HİJYENİK YETİŞKİN/HASTA BEZİ GELİŞTİRİLMESİ VE ÜRETİMİ

Mikroorganizmalar nemli ve besin olan ortamlarda çok kolaylıkla üreyip insanlarda hastalıklara sebep olabilirler. Bu nedenle boşaltım sisteminde sorun yaşayan yetişkinlerin kullandıkları hasta ve/veya yetişkin bezleri de primer ve seconder patojenler için elverişli bir üreme ortamı oluşturur.

Bu çalışma kapsamında hasta ve/veya yetişkin bezlerinin kullanımı sırasında hijyenik özellik kazandırılması hedeflenmiştir. Antimikrobiyal özelliğe sahip bor bileşikleri ve pluronik moleküllerinin formüle edilerek hasta/yetişkin bezlerinin üretilmesi planlanmaktadır.

Bu amaçla hasta bezi kullanımı sırasında o bölgede kolonize olabilen patojenik mikroorganizma türleri belirlenmiş ve bu mikroorganizmalar üzerinde antimicrobiyal testler yapılmıştır. Bor bileşikleri ve bor türevlerinin pluronik kombinasyonları *in-vitro* koşullarda antimikrobiyal testleri yapılmıştır. Belirlenen formülasyonlar yetişkin ve/ veya hasta bezlerinin tasarımlarında kullanılmıştır.

Yaşlı nüfusu ilerleyen yıllarda artacağından yetişkin/hasta bezinin kullanımı da artacağı düşünülmektedir. Bu nedenle günümüzde kullanılan bezlerin konfor, sızdırmazlık gibi özelliklerinin iyileştirilmesi ve bu açıdan mevcut bezlerden daha iyi performans sağlayan tasarımların yapılması gerektiği öngörülmektedir. Bu nedenle, bu çalışmada hasta ve/veya yetişkinlerde kullanılacak, hasta bezi üzerinde oluşan patojenik mikroorganizmaların inhibe edilmesi amaçlanmıştır.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

ABC	ATP binding cassette transporter
APD	Adult/Patient diaper
BA	Boric acid
DD	Diaper dermatitis
DOT	Disodium octaborate tetrahydrate
EO	Ethylene oxide
ICS	International Continence Society
MIC	Minimum Inhibition Concentration
NHANES	National Health and Nutrition Examination Survey
OI	Overflow incontinence
pF127	Pluronic F127
pF68	Pluronic F68
PO	Propylene oxide
SAP	Superabsorbent polymer
SPT	Sodium pentaborate tetrahydrate
STD	Sodium tetraborate decahydrate
SUI	Stress incontinence
UI	Urinary incontinence
UUI	Urge incontinence
ZB	Zinc borate

1. INTRODUCTION

Diapering of infants who are not potty trained is as old as human history [1]. In millennia, families who live in warm weather areas did not need any dress to wrap their infants so they only clean babies waste if they contaminate their food or working areas. In colder regions, to keep infants warm and secure, mothers tied up babies from neck to toe with swaddling clothes. This can be accepted as the first diaper type in history. In 1930s, Europe and North American researchers developed different types of disposable diapers. In Europe, Swedish paper company produced cellulose sheets (35 cm x 40 cm). Parents used these sheets between two fabric materials for diapering purpose. During the 1940s, diaper technology started to evolve. Nearly 1000 patents and design contributed to the evolution of diaper until 1990s. After 1990s, disposable pads with superabsorbent polymer (SAP) started to be produced by manufacturers [1]. Lots of companies started to produce their own diaper design to make diaper thinner and more compelling for consumers. Today, companies are developing and designing premium diaper segments which have leakage control parts (curved elastic leg openings, waste barriers), comfort parts (cloth-like covers and breathable material) and hygienic parts (rash guard surface) to make them more appealing to consumers and their children. After these development companies produced their products in three types of diaper; baby diapers, underpants, Adult/patient diapers (APD) [2].

APDs are needed for patients having urinary incontinence. Urinary incontinence (UI) is the most common problem which is defined by the International Continence Society (ICS) as a condition where involuntary loss of urine [3]. Incontinence problem can be permanent, which is related to neurological damage (sclerosis, spinal cord injury and stroke) or can appear temporarily due to food and drinks that irritate the bladder. Also, traffic accidents, after delivery of a baby or surgical operation may lead bladder weakness and may cause UI. According to Alayne et al. conducted a survey of 17850 adults who are 20 years or older, the prevalence of urinary incontinence (UI) in women 51.1 per cent and 13.9 per cent in men [4]. According to report from the National Health and Nutrition Examination Survey (NHANES) on 23 million women, 38 per cent of them complain about the UI. Another survey in England about the UI, there are 3.5 million people have urinary incontinence problem reported officially. Researchers consider that these surveys do not

give realistic data, because 10 million people have trouble about UI problem, but they don’t inform officially because of being ashamed from incontinence. UI problems are basically divided into three types; stress incontinence, urge incontinence, overflow incontinence.

Stress incontinence (SUI) is involuntary leakage of on effort or on sneezing or coughing [5]. SUI has significant effects on women's life quality, but the most women do not categorize SUI as a major problem for their life. Therefore, they do not take any medical support for this problem. According to survey of age related SUI risk, the prevalence of SUI steadily increases with age of 65 and after [6,7]. SUI is more frequently appeared in pregnancy condition and parous women compared to nulliparous women. Increasing body mass index has also relationship with SUI, as increasing body weight causes chronic damage to the pelvic floor musculature. Smoking and lung diseases are another risk factor for SUI. The survey on women showed that women who smoked previously have a risk factor for SUI 2.2-fold increase, and women who are currently smoking have a risk factor for SUI 2.5-fold increase compared to non-smoking women [8].

Urge incontinence (UUI) is the complaint involuntary leakage with immediately preceded by an urge to urinate. Another cause of UUI problem is detrusor overactivity [9]. When the patient whose bladder filled with liquid demonstrates spontaneous detrusor contraction so this results in detrusor overactivity. Especially, involuntary contraction of detrusor muscles may have relation with neurological conditions (multiple sclerosis, cerebrovascular accident, spinal cord injuries) and condition termed as neurogenic detrusor overactivity. UUI problem may not only appear with neurogenic problems, but also related with surgical interventions which cause denervation of detrusor or excitability of person [10]. Such condition is called as idiopathic contraction [5]. Figure 1.1. shows that the urge incontinence is depended on ages. Women with urge continence sometimes do not demonstrate detrusor over activity. For diagnosis of UUI in women, pad tests are used to quantify urine lost before and after leakage provocation. Pad tests are divided as short-term tests which can be performed in office condition and long-term tests which are usually performed at home for 24 to 48 hours [5]. Short-term tests are performed with full bladders before starting an exercise or routine work.

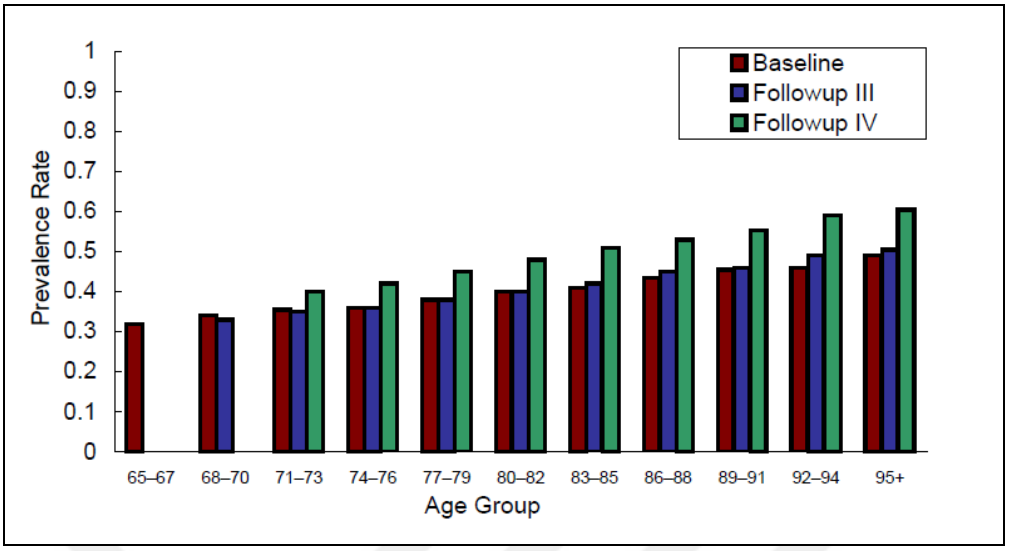

Figure 1.1. Prevalence of urge incontinence by ages. Follow-up shows include responses 3 and 6 years after baseline [5].

Overflow incontinence (OI) problem appear when bladder become too full and when it cannot be fully emptied by the person. This condition may cause bladder obstruction or injury. OI is also caused by bladder dysfunction or obstructed urinary outflow. Most common cause of overflow incontinence is diabetic neuropathy. In addition, chronic overflow is a common problem in men because of prostatic hyperplasia (Figure1.2).

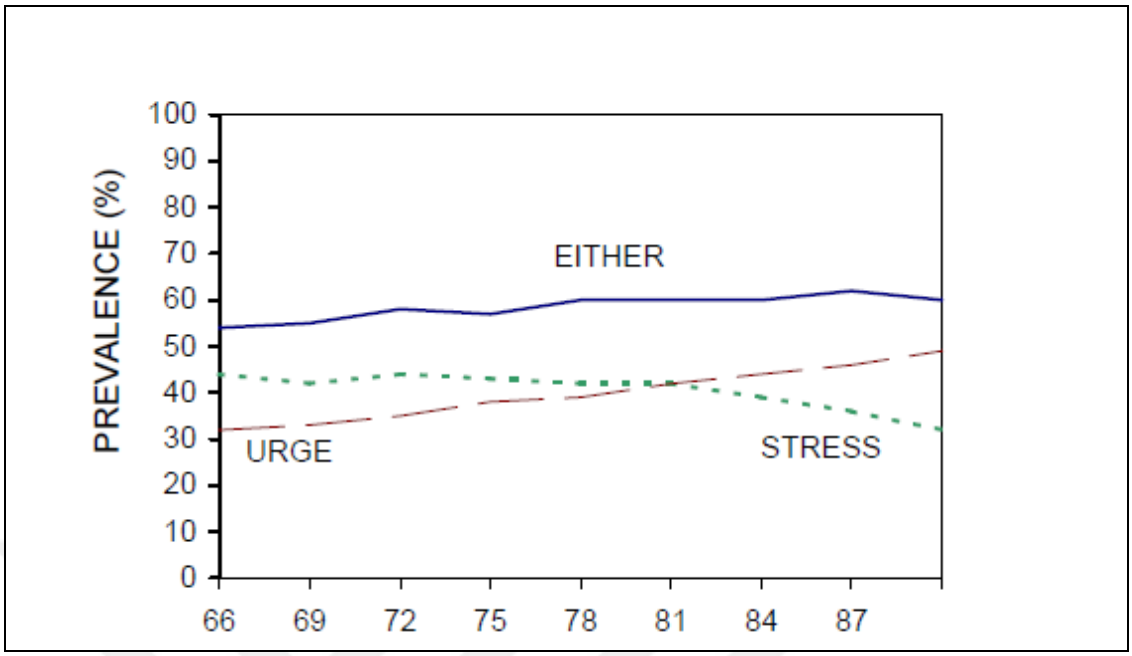

Figure 1.2. Prevalence of urinary incontinence by age [5]

Disposable diaper companies have started to produce new designs to serve a more comfortable life for patients with UI and other types of problems (Figure 1.3). Disposable APDs producers construct their APD design on critical factors for the protection of patient safety; high suction performance, prevention of bad smell, and in addition, leakage proof are important factors for the production of high quality AD diapers. High suction performance is a measure of absorbing time of urine by product [1]. APDs with SAP are new designs to decrease absorbing time of the diaper. SAP materials are hydrophilic hydrogel material that can absorb huge amounts of water or aqueous solutions. They can absorb deionized water 1,000-100,000 per cent (10-1000 g/g) of their own mass [2]. After SAP absorbs water and turns into gel form even under pressure, they can hold the water or urine. Water absorption capacity (WAC) is the most important characteristic of SAP. WAC can be measured by different ways (volumetric method, gravimetric method, spectroscopic method and microwave method), but there isn't any universal method for measurement. Moreover, prevention of bad smell is an essential feature for hygienic diaper technology. Waste barriers stuck urine and feces odor between skin and diaper after usage of diaper. Backsheet and topsheet of diapers are coated with non-woven fabrics, and these parts of the diaper protects leakage from diaper when fulfill with urine and feces. Although, a lot of APD diaper designs and patents are made to develop more functional disposable diaper

and protect the patient safety, there are still serious health problems concerning usage adult diapers. Despite, all urine and aqueous solutions are uptaken by SAP containing-disposable diaper, top sheet of diaper may still remain moist. Wetness of the diaper area affects patient's skin and cause serious health problems.

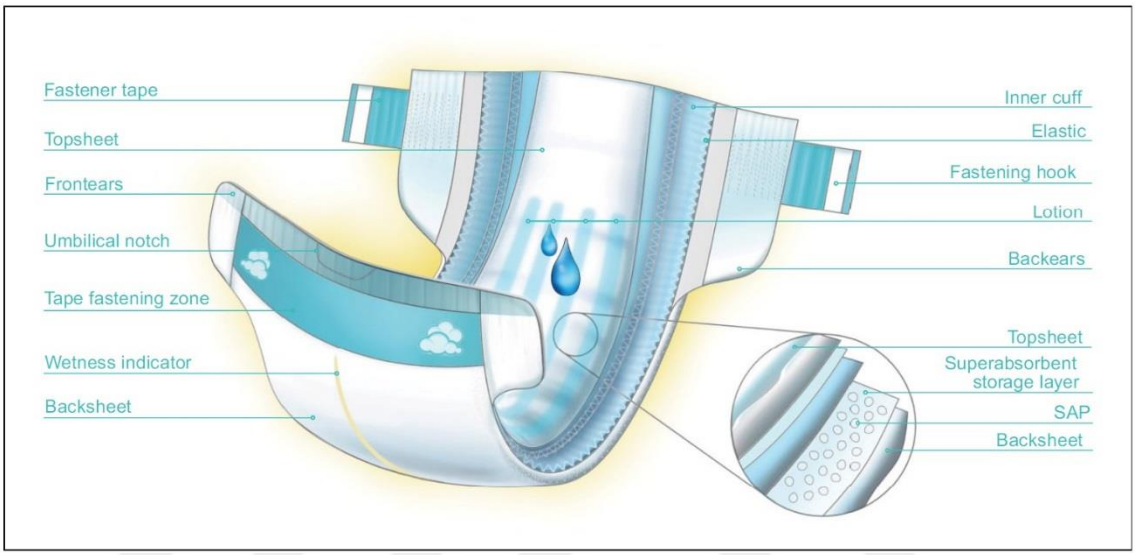

Figure 1.3. Modern disposable diaper [13]

1.1. DIAPER DERMATITIS

Diaper dermatitis (DD), is one of the most common dermatologic disorder of adults. DD can be subdivided into primary diaper dermatitis, an acute inflammation of the skin in the diaper area with a multifactorial etiology, and secondary diaper dermatitis. The most important factors in the development of primary diaper dermatitis are: (i) water/moisture, (ii) friction, (iii) urine, (iv) feces, and (v) microorganisms (Figure 1.4) [3]. Moisture in the diaper area cause adverse effects on the skin, such as maceration, are likely to be the most important factors in the development of the DD. The moisture (i) makes the skin more fragile, increasing with frictional damage; and (ii) undermines the ‘barrier function’ of the skin, with resultant increases in irritant chemical penetration and pathogen replication [4]. The erosive effect of fecal enzymes (urease, proteases and lipases) which activity increase with elevated pH and weakens epidermal integrity. Moreover, urine is another problem

which can increase the permeability of diapered skin to irritants and can directly irritate skin when exposure is prolonged. Urine can also elevate wetness and pH level therefore weakens epidermal integrity [5]. As a result of this, it causes maintenance of the pathogenic microflora on the skin surface and gives a chance to microorganism to colonize in a diaper and skin area.

When the clinical appearance of primary dermatitis investigated, it has variety presentation, such as granuloma gluteale infantum, chronic diaper dermatitis, jacquet's diaper dermatitis (dermatitis syphiloides posterosiva) and irritant contact dermatitis [3]. Granuloma gluteale infantum is an uncommon disorder, appear as 0.5-four cm nodules on the buttock, lower abdomens and penis, and can be classified as a type of irritant diaper dermatitis [6]. Jacquet's diaper dermatitis is another variant of primary diaper dermatitis. It is described as a severe noduloerosive lesion presentation in the diaper area. It can appear after consequences of severe diarrhea or cleaning the vaginal area with toilet paper when the paper cause eruption on skin [7]. Chronic diaper dermatitis is commonly seen in infants with chronic diarrhea. Another variation of primary dermatitis is Irritant contact dermatitis which appears as erythema often with maceration, erosions and ulceration. Problems commonly are seen in gluteus maximus, genitalia and lower abdomen. Patients' diet or medicine (especially antibiotics) can directly affect irritant contact dermatitis level when wearing APD. After nutrients are digested and thrown as urine or feces, diaper uptakes substances and direct contact of these substances with the skin may cause serious irritant contact dermatitis in patients. Exposure time, pH level, temperature in the diaper area, wetness of diaper and duration of contact with skin, interaction with substances are counted as essential causes of irritant contact dermatitis.

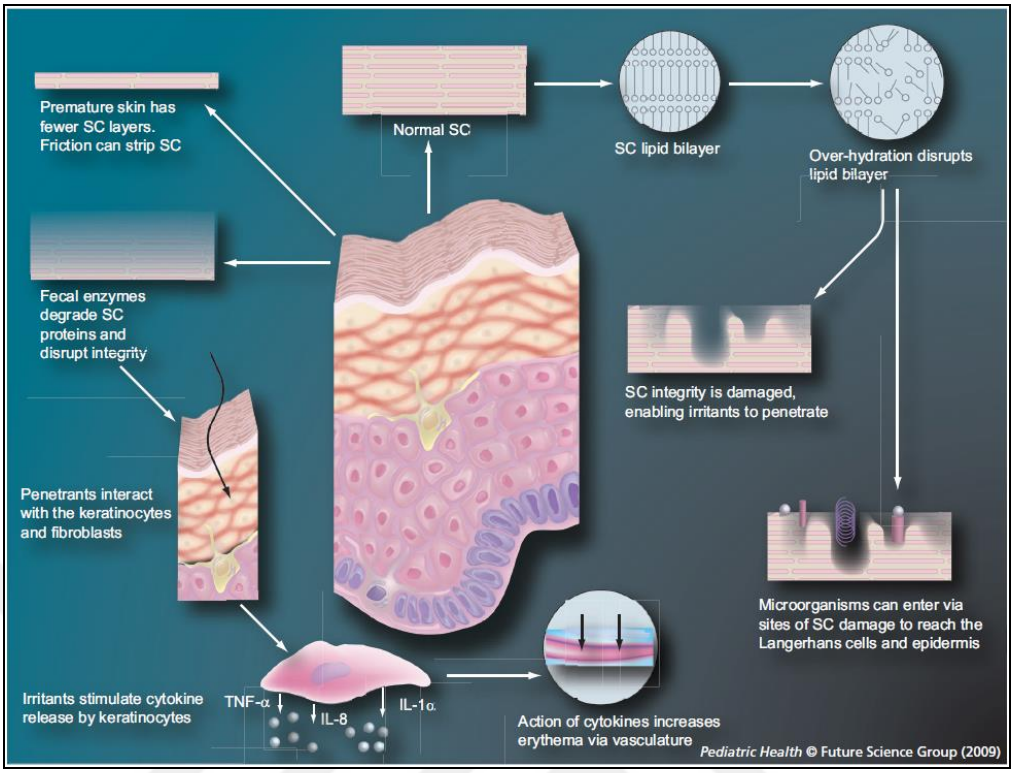

Figure 1.4. Effects of diaper to skin barrier [17].

Secondary dermatitis is another main DD problem related to inflammatory mechanism and infections in diaper area. When focused on inflammatory eruptions on the diaper area, there are variety of inflammation disorders appearing on the skin such as milliria rubra, seborrheic dermatitis and allergic contact dermatitis. Milliria rubra appears as red papules or fragile vesicles. It is termed as blockage of the eccrine ducts because of toxin production of *Staphylococcus epidermidis*. In immobilized persons, milliria rubra most commonly occurs on the back and on diapered area [8]. Especially, milliria rubra occurs at elasticized openings of the diaper or the tape line of diaper obstructs the discharge of eccrine secretions [9,3]. Although, cause of seborrheic dermatitis is unknown, many exogenous factors have been cited as possible contributors to the development of this disorder [10]. Generally, seborrheic dermatitis occurs on marginated area of erythema and scale in diaper area. Another secondary diaper dermatitis problem is allergic contact dermatitis, and it is defined as the most common serious problem in younger people because the activity of immunity is stronger than older people (Figure 1.5). Allergic contact dermatitis is type four hypersensitivity reactions. Diaper caused irritation on the skin causes activation of

upregulation and recruitment of chemokine genes. Respective gene expressions regulate T-cell effector cytokines productions [11].

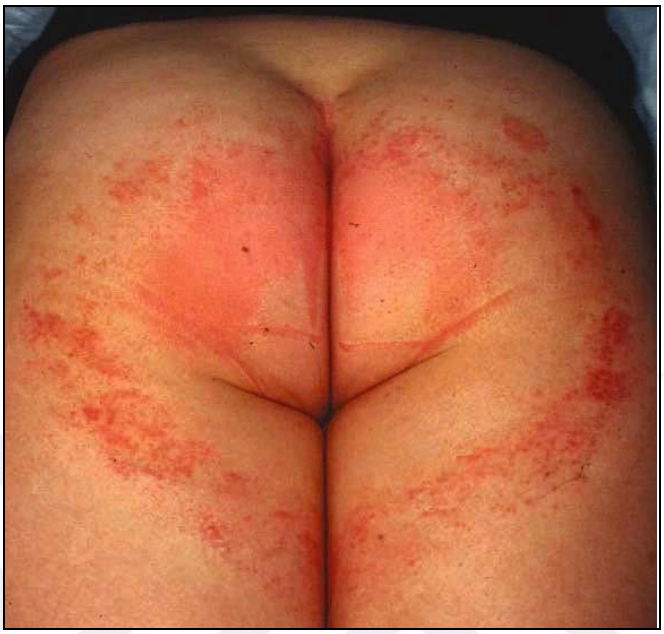

Figure 1.5. Allergic contact dermatitis is one of the most serious problems in young diaper consumers [21].

1.1.1. Fungal, Yeast and Bacterial Infections in Diaper Area

Diaper dermatitis caused by *Candida* species is the most common secondary dermatitis. Generally, *Candida albicans* is the most seen pathogenic yeast in the diaper area. The source of *Candida* species can be the upper or lower gastrointestinal tract or on care provider. If diaper dermatitis is present for more than 3 days on the skin, colonization of *C. albicans* increases on genital creases, abdomen and genital area. After colonization of *Candida* species on marginated zones, these areas show erythema with papules, pustules, and satellite papules. It is suggested that *C. albicans* antigens play important role in development of skin lesions [12]. In development phase of *Candida* species, candidal diaper dermatitis can become invasive and life threatening [13]. Although, *C. albicans* is the primary reason for diaper dermatitis, other bacteria and yeast species are also pathogenic in diaper dermatitis. The most frequently isolated yeast species have been *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. kefyr* (*Kluyveromyces marxianus),*

C. pulcherrima, C. guilliermondii and *C. zeylanoides*. Bacterial infections also occur in diaper area. Impertigo which is generally caused by *Staphylococcus aureus* appears in the diaper area. It appears as perifollicular erythematous and pustules that may spread to thighs and abdomen. Sometimes, other bacteria species can cause folliculitis. *Escherichia coli* is another pathogenic species which is the most abundant bacterial species in human intestinal microflora but highly adopted *E. coli* can gain virulence factor attributes. The diaper contaminated with urine and feces helps maintenance of pathogenic *E. coli* to cause diarrheal, urinary tract infection or sepsis [14]. *Pseudomonas aeruginosa* is the main cause of ecthyma gangrenosum, a skin infection and cutaneous lesion of systemic infection problem in the diaper area. Some cases showed that impetigo like psoriasis disease caused by *Bacillus cereus*. Additionally, *B. cereus* can sometimes cause seborrheic dermatitis in the skin. *Acinetobacter baumannii* is another pathogenic bacteria and specifically targets moist tissues. When skin and soft tissues infected with *A. baumannii*, it may cause sandpaper-like presentation on the skin area [15].

Today, combating with diaper dermatitis is an important problem, and some precaution strategies are taken for the prevention from diaper dermatitis. i) Maintenance of dryness diaper can be a preventative strategy for prevention of maceration of stratum corneum. ii) Exposure time of diaper to skin time after usage of diaper is the main precaution strategy for prevention. Frequent changes of diaper may minimize urine and feces exposure to skin. iii) Maximizing diaper-free time is another recommended strategy to prevent diaper rash. iv) Patient or diaper user's buttocks sides cleaning with alcohol-free cleansing products and non-woven disposable wipes may help removal of destructive enzymes from skin surface and also contribute to maintenance of the pH level of the skin and diaper area [16].

Medical treatments are another prevention strategy to decrease pathogenic infections ameliorating DD. For instance, antimicrobial agents have been used to treat DD. Miconazole nitrate containing ointments and pastes used to decrease erosion [17]. Mupirocin is another antibacterial agent used to prevent DD, but it has to be applied three-four times in every diaper change. Parfenac lipid ointments have been used as an effective treatment for diaper rash [3]. In one study, oral zinc has been found as an effective treatment to decrease microbial growth [18]. Although there are encouraging, promising treatments and strategies used for the treatment and prevention of DD, they remain inadequate due to being expensive and do not completely inhibit development of

microorganism in the diaper area. Therefore, new therapeutic strategies and antimicrobial agents are urgently needed to decrease pathogenic development in the diaper area.

1.2. BORON

Boron has metal and nonmetal physical properties and isn't found naturally in elemental form. Boron is almost always found to be linked with oxygen. It is also found in nature in the form of inorganic oxides and there are 200 different types of boron mineral found in nature. These boron minerals are called as borates and the main forms of boron are boric acid, borates and borosilicate minerals.

Boron is required and essential mineral for growth of plants, diatoms and marine algal flagellates [19,20]. More recently, boron was shown to be required mineral for proper development of animals and also, some research revealed that boron is also necessary for health of human [21]. Bio-essentiality of boron in animals and humans has taken a long time to reveal by researchers because boron is the most common mineral in the environment and it is so difficult to remove from foodstuff or drinking [21]. Today, researchers found that boron plays a vital role and influence metabolic processes in microorganisms, plants, animals and human. It plays an essential role in quorum sensing, borate-sugar di-ester complex is crucial for cell-cell communication, in marine bacteria. It is also required for plants as nutrition, fertilization, fruiting and seed production in plants[22]. The most important properties of the borates are that it shows biocide properties against organism or microorganism. First natural boron-containing natural products are characterized in antibiotic form which are aplasmomycin [23], boromycin [24] and tartralon B [25] (Figure 1.6). The most common registered boron containing compounds, used as antimicrobial agent, are boric acid, disodium octaborate tetrahydrate, sodium pentaborate tetrahydrate, sodium tetraborate decahdyrate, and zinc borate, which are most common biocides and antimicrobial minerals in industry.

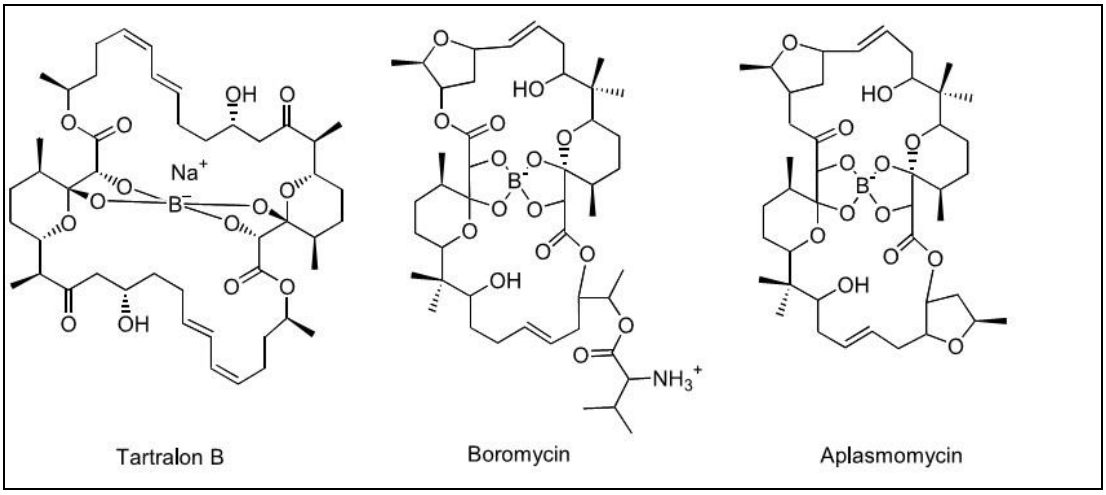

Figure 1.6. First natural anti-microbial boron-containing products and their chemical formulas [34].

1.2.1. Boric Acid (BA)

Boron is most commonly found in the form of BA in nature and associated with orthoboric acid ($B(OH)_3$) (Figure 1.7). It is also found in nature as a mineral sassolite form that is the predominant form in natural waters [22].

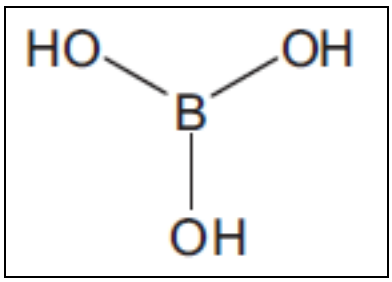

Figure 1.7. Boric acid is water soluble borate-type mineral which acts as Lewis acid

While BA is an essential nutrient for plant, animal and fungi, high concentration of BA shows toxic effects on microorganism. Aqueous solution of BA is being used as a cleansing agent for the ears or skin cleaner of animals [26]. Additionally, BA is used as a common pest control agent to control of cockroaches. Bacteriostatic effects of BA was firstly described by Porter and co-workers in the research about preservation of urine

sample in 1969 [27]. After that, bactericidal activity of BA was also reported in 1971 [28] but infection treatment using BA as a therapeutic agent, especially for treatment of yeast infections, were studied several years later [29]. Today, BA is being used as an alternative treatment of candidal vulvovaginitis that is a common *Candida* sp. infection problem in women [30]. According to Sobel and co-workers, candidal vaginitis related to *C. glabrata* was treated with BA (600 mg/day) in gelatin capsules for 14 days, and the results showed that 77 per cent of patients (20 of 26 patients) clinically cured and 81 per cent of patients showed improvement in their treatments [31]. Seta and co-workers showed that 71 yeast strains which were isolated from patients with candidal vaginitis were inhibited with 6175 mg/ml BA, and 46 per cent of *C. albicans* inhibited by boron treatment [8]. In another study conducted by Martin and co-workers revealed that 0.4 per cent BA caused delocalization of the contractile actinomysin ring in *Saccharomyces cerevisiae* resulting cell wall synthases disruption [32]. Moreover, BA exhibited antibacterial activity against *S. aureus* ATCC 25923, *Acinetobacter septicus* DSM 19415, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853 strains. The antibacterial activity of BA range was found between 3.80 mg/ml and 7.60 mg/ml [33]. Taken together, BA is a promising antimicrobial agent which can be used in microbiological disease treatment and as an industrial antimicrobial agent for cleansing products.

1.2.2. Sodium Tetraborate Decahydrate (STD)

Sodium tetraborate, also known as Borax-$Na_2B_4O_7$ or $Na_2O.2B_2O_3$, is available in different crystalline and glass forms [34]. Crystalline form (most common α-crystalline form) of Borax is generally prepared for research purposes also known as anhydrous borax in industrial researches [35]. This crystal form of boron generally is being used as biocide and wood protection agent for rot fungi. STD has alkaline pH of 9.5 and it is good scouring compound used as bleaching agent. Additionally, because of its alkaline structure, it is commercially used as antimicrobial additive in soap for cleaning of hands and body from microorganism. According to Sarah and co-workers, STD powder exhibited effective anticandidal activity against yeasts [36]. It is also a useful antimicrobial additive agent for hospital grade disinfectants which is commercially used as a medical instrument cleanser [37]. Another research on STD showed that it can be used for treatment of acne, which is caused by overactivity of sebaceous glands of the skin [38]. It has also been used as

commercial adhesive. Starch and dextrin adhesives are the most common commercial products and STD used as a cross-linker, biocide agent and also for increasing viscosity of adhesive [22].

1.2.3. Disodium Octaborate Tetrahydrate (DOT)

Disodium octaborate tetrahydrate, $Na_2B_8O_{13}.4H_2O$ or $Na_2O.4B_2O_3.4H_2O$, is amorphous and white powder which easily dissolves in water without the requirement of temperature change. DOT commonly contains 66.3 (wt/wt) per cent B_2O_3. If B_2O_3 composition changes inside of the DOT, water solubility of DOT also decrease with the composition ratio (Figure 1.8) [39]. DOT is commercially used in industry as a cleansing agent. For instance, DOT aqueous solutions also being used as antimicrobial additive in cleansing products for kill dust mites on carpet [40]. DOT is also being used as wood preservative to protect wood against termites, wood boring beetles and carpenter ants [41]. There is a lot of patented cleansing products containing DOT being used in industry, as Jeffrey and co-workers developed surface cleansing method using 0.05 per cent to five per cent DOT for making bactericidal and insecticide area [42]. According to Sayın and co-workers investigating the antibacterial effect of DOT, one-three mg/ml DOT has bactericidal and bacteriostatic effects on pathogenic bacteria (*S. aureus*, *Aeromonas hydrophila*, *P. aeruginosa*, *Brucella melitensis)* [43].

Temperature, °C	Solubility, disodium octaborate tetrahydrate, wt %	B_2O_3 concentration in saturated solution, wt %	
		Disodium octaborate tetrahydrate	Borax
0	2.4	1.6	0.7
10	4.5	3.0	1.1
20	9.5	6.3	1.7
30	21.9	14.5	2.6
40	27.8	18.4	4.1
50	32.0	21.2	6.5
60	35.0	23.2	11.1
75	39.3	26.0	14.7
94	45.3	30.0	21.0

Figure 1.8. Solubility of disodium octoborate tetrahydrate with different composition of B2O3 [45].

1.2.4. Sodium Pentaborate Tetrahydrate (SPT)

The most common hydrated form of Sodium Pentaborate structural formula is $NaB_5O_8.5H_2O$. It exists in the mineral form known as sborgite. SPT can easily crystallize in solutions, but solubility of SPT in water five per cent and also low in other organic solvents at room temperature (Figure 1.9) [35]. SPT is another alternative borate mineral for pesticide and fungicide agent used in industrial products.

Solvent	Temperature, °C	Solubility, wt %		
		$B(OH)_3$	$Na_2B_4O_7{\cdot}5H_2O$	$Na_2B_4O_7{\cdot}10H_2O$
glycerol, 86.5%	20	21.1	47.1	
glycerol, 98.5%	20	19.9	52.6	
glycerol	25	17.5		
ethylene glycol	25	18.5	41.6	31.2
propylene glycol	25	15.1		21.9
diethylene glycol	25	13.6	18.6	10.0
mannitol, 10%	25	6.62		
methanol	25	173.9[a]	19.9	16.9
ethanol	25	94.4[a]		
n-propanol	25	59.4[a]		
n-butanol	25	42.8[a]		
2-methylbutanol	25	35.3[a]		
isoamyl alcohol	25	2.39		
acetone	25	0.6	0.60	
methyl ethyl ketone	20	0.7		
ethyl acetate	25	1.5	0.14	
diethyl ether	20	0.008		
dioxane	25	~14.6[a]		
pyridine	25	~70[a]		
aniline	20	0.15		
acetic acid, 100%	30	6.3		

[a]Solubility values are in g/L.

Figure 1.9. Solubility of boric acid, sodium tetraborate decahydrate and sodium pentaborate in organic solvents [30].

1.2.5. Zinc Borate (ZB)

Zinc borate has $2ZnO_3B_2O_3.7H_2O$ structural formula. Several crystalline hydrated and anhydrous zinc borates are common industrial products [44]. The most common usage of zinc borate is as a fire-retardant agent with synergists for polymers. ZB is being used, like other borate types, as a preservative for engineered wood products and wood-plastic composites. There is a lot of patented products containing ZB being used as antimicrobial additive in plastics such as ZB can be a useful additive for polyacetal resins to make plastic corrosion resistance against bacteria (*E. coli* and *S. aureus*) and fungi (*Aspergillus niger*, *Cladosporium cladosporioides* and *Trichoderma sp.*) species [45]. Another product which is ceramic glaze containing ZB also exhibited effective antibacterial activity against *E. coli* [46]. It is also used as fungicides in coatings. Moderate concentration of ZB can inhibit growth of wood-destroying fungi and wood boring insects (termites, carpenter ants and beetle larvae).

1.2.6. Boron Toxicity

Boron compounds' toxicity have been tested on several species for developing proper usage by human and also for safe handling. Researches about boron toxicity tested in vertebrate species such as fish, frogs and mammalian species such as rats, mice and dogs. While evaluating toxic effects of boron, different dosage application applied to animals as acute and chronic by researchers. The experiment which conducted on dogs showed that oral LD50 in dog was measured as >3980 of boric acid/kg and >6150 mg of sodium tetraborate decahydrate/kg. There are no other adverse effects were observed by researchers [47]. Another research investigating the effects of ZB on rats showed that the acute oral LD50 of ZB was greater than 10,000 mg/kg in albino rats and there were no adverse effects observed [22].

The most important toxicity for boron and borates is dermal toxicity of diaper usage. Results revealed that acute dermal toxicity is low for borates: LD50s were >2000 mg/kg for boric acid, boric oxide, disodium octaborate tetrahydrate, sodium tetraborate decahydrate, and sodium tetraborate pentahydrate, and >10,000mg/kg for zinc borate (Table 1.1).

Table 1.1. Inorganic boric acid and borates compound LD50 values on rats [58]

Boron Compound	**Chemical formula**	**LD50 (mg/kg) in rats**
Boric acid	H_3BO_3	2660-4100[48]
Boric oxide	B_2O_3	>2000[49]
Disodium tetraborate decahydrate	$Na_2B_8O_{13}.4H_2O$	4500-6000[48]
Zinc Borate	$2ZnO.B_2O_3.3.5H_2O$	>10,000[49]

Boron compounds were also evaluated for their long term exposure toxicity and chronic side effects on species. There was not any sign for that boron compounds cause genetic mutations, cancerogenic and cancer-causing [50]. According to Linder and co-workers, exposure of boron at low doses (175 mg/kg) body weight (bw) cause reversible inhibition of spermiation but single dose exposure (2000 mg/kg boric acid) had no such effects.

Moreover, a group of dogs' dietary was adjusted to intake BA or STD at doses up to 10.2 mg B/kg bw/day (62.4 mg boric acid/kg bw/day and 84.7 mg STD/kg bw/day) and there was no adverse effect observed [50]. In another study, dogs were fed with 39.5 mg B/kg bw/day (233.1 mg boric acid/kg bw/day and 373.2 mg sodium tetraborate decahydrate/kg bw/day) and no observable adverse effect levels were observed. Boron was not reported to be absorbed across intact skin. Friss-Hansen and co-workers research showed that there was no absorption through the skin when 22 infants treated with ointment containing three per cent BA (approximately 16 mg B) for four-five days [51]. In addition, BA was applied topically to forearm of volunteers for four hours and there was no increase in urinary boron [52]. Absorption tests on animals (rat and mice) have shown the similar results. Rat with intact skin was treated with ointment containing three per cent of BA and it was observed that skin did not absorb significant levels of boron [53].

1.3. PLURONICS

Poloxamers are biological active polymers and they are referred as Pluronic block polymers in medical and research applications. Pluronics are triblock copolymers consisting of a hydrophobic propylene oxide unit (PO) which is flanked by two hydrophilic ethylene oxide (EO) units at each end(Figure 1.10) Different types of Pluronics can be characterized according to number of EO and PO units [54].

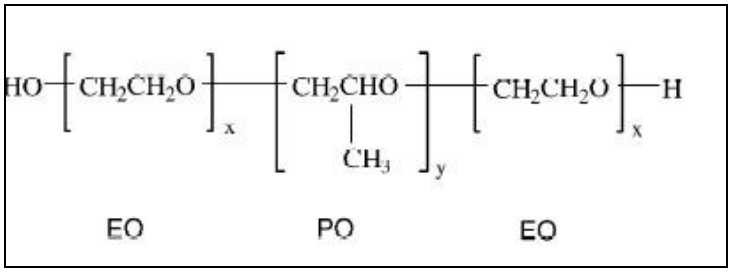

Figure 1.10 Pluronic blocks arranged EO-PO-EO structure. In the equation, x and y units can be altered according poloxamer length [58].

They can be synthesized with the initiation of PO polymerization reaction followed by the addition of EO to the chain and again reaction ends with PO blocks. After the polymerization reaction is ended, generally physical appearance of pluronics has spherical

types and called Pluronic micelle. Different types of Pluronics are being used in the industry as solubilization, coating, wetting agents and antimicrobial agent carrier. It has well biocompatibility and the ability to deliver the therapeutic agents such as drugs, nucleic acids, growth factors to the targets [55]. Additionally, Pluronics developed for drug carrier to pass resistance mechanism and also increasing effect of drugs [55]. Also, drug carrier Pluronic polymers are required for drugs having poor stability, low water solubility and severe side effects [56]. There are two major types of Pluronic used as an antimicrobial and anticancer drug carrier, pF68 and pF127.

1.3.1. Pluronic F68 (pF68)

Poloxamer 188 having a chemical formula of $PEO_{76}PPO_{29}PEO_{76}$, and a trade name of Pluronic F68, can attach to cell membrane and prevent cell aggregation along with protecting the cell from mechanical stress. For example, pF68 is being used as mammalian or insects protective agents from sparging (supplying oxygen in bioreactor that can damage cells) [57]. Additionally, they are involved in antithrombotic activities, hemorheological activities, phagocyte activity in the cell. According to Echevarria and co-workers, encapsulation of amphotericin B with nanosphere polymer of pF68 increased the activity of antifungal agent against microorganisms [58]. Other study conducted by Kamboj and co-workers on anti-cancer drug paclitaxel, which loaded nano polymer with pF68 formulation for treatment breast cancer. Results showed that paclitaxel loaded with pF68 was found to be more efficient in impeding breast tumor development compared to equivalent of doses paclitaxel [59]. Another research by Cai and co-workers revealed that curcumin - pF68 conjugation showed 1.95- fold more cytotoxicity than free curcumin against cancer cell lines [60]. Therefore, pF68 will be a potential antimicrobial agent carrier for increase inhibition of microorganisms in diaper area.

1.3.2. Pluronic F127 (pF127)

Poloxamer 407 having the chemical formula $PEO_{100}PPO_{65}PEO_{100}$, known commercially as pF127, contains ∼70 per cent polyoxyethylene units and 30 per cent polyoxypropylene blocks. It has low toxicity, high compatibility with other chemicals, especially medicines

and easily dissolve in aqueous solutions [61]. They are being used as creating anti-adhesive surface for bacteria on hydrogel contact lenses [57]. Pluronic 127 has potential usage, like pluronic 68, as topical drug delivery carrier since it shows reverse thermal gelation behavior. These features of pluronic 127 has good for administration drug routes such as oral, topical, intranasal, vaginal and parental routes [62].

1.4. AIM OF THE STUDY

Lots of precautions are taken for the prevention of DD and diaper microorganism related diseases, but the prevention strategies are not practical and permanently solving problem for the microorganism related diseases. Therefore, new diaper designs are urgently needed to solve or prevent DD-related problems. The aim of the current study was to design antimicrobial adult/patient diapers with boron derivatives and poloxamer (pF68 and PF127) combinations. Additionally, it was designed to prevent microorganism-related diseases and therefore, to decrease the prevalence of DD. The current study will be held to i.) investigate the antimicrobial effects of boron derivatives and their combinations with pluronics (pF68 and pF127) against pathogenic microorganisms causing DD, and ii) investigate the antimicrobial effects of APD diaper containing boron and pluronics combinations on microorganism which may colonize in the diaper area.

2. MATERIALS AND METHODS

2.1. IDENTIFICATION OF PATHOGENIC MICROORGANISM

The microorganisms including *E. coli* ATCC 10536, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442, *A. baumannii* wild type, *B. cereus* ATCC 1778, *C. glabrata* wild type, *C. albicans* ATCC 10231, *K. marxianus* wild type, *A. niger* ATCC 16404 were provided from Yeditepe University Microbiology Culture Collection, identified using microbial identification system (MIS) and rDNA based sequencing. Briefly, four types of solution were prepared before MIS method applied to the microorganism. Solution one was used for saponication, prepared with sodium hydroxide (NaOH, #67-56-1, Sigma-Aldrich, USA) (45 gr), methyl alcohol (CH_3OH) (150 ml), distilled water (150 ml). Solution two was used for methylation, HCI (6.00N) (325 ml) and methyl alcohol (CH_3OH) (275 ml) was used. Solution three was prepared for extraction, in which hexane $CH_3(CH_2)_4CH_3$ (#110-54-3), ethly-tert-butyl ether (#637-92-3) (MTBE) (200 ml) were mixed. Solution four was prepared for base wash, where NaOH (10.8 gr) was dissolved in 900 ml dH_2O.

Firstly, microorganisms were cultured in Tryptic Soy agar (TSA) for bacteria and Sabouraud dextrose agar (SDA) for the yeast and fungi at 37 ºC for overnight. Microorganisms were inoculated in solution one (one ml) and incubated 100°C in water bath for 30 min. Then, Solution two (two ml) was added to each sample and incubated at 80ºC for 10 min. Then, samples were put in ice-water to add methyl ester bonds to fatty acids. After that, Solution three (1.25 ml) was added to the experimental samples. After organic and acidic phases were separated, acidic phase was transferred to new tubes. Solution four (three ml) were added and the tubes were incubated for 10 minutes at room temperature. After phase separation, down phase was taken and analyzed with Agilent Technologies 6890N network GC system.

For rDNA-based sequencing analyses, yeast and fungal species' genomic DNA were isolated according to the protocol described in the literature [63]. Briefly, 700 µl homogenization buffer (10 mM Tris, pH 8, 2 mM EDTA, 0,4 mM NaCI) was added into each yeast and fungi strain containing tubes and vortexed for 1 min. After that, 40 µl of 20 per cent SDS was added and shaked heavily and incubated one hour at 60 ºC in water bath.

Then, 300 µl of 6M NaCI was added to each samples and vortexed for 30 sec. The tubes were centrifuged at 1000 x g for 30 min and supernatants were transferred to new tubes. One volume of isopropanol (about 700 µl) was added to each sample and shaked heavily. Then, the tubes were incubated one hour at -20 º C and centrifuged at 10,000 x g for 20 min. After that, supernatants were removed and 500 µl of (70 per cent) ethanol was added to the samples and the tubes were incubated for 30 min at room temperature. Finally, 50 µl of dH_2O was added to each samples to dissolve DNA in water.

After genomic DNA was isolated from yeast and fungal species, Intron PCR (#S25266) kit was used to amplify DNA with the universal primers; ITS2 5'-GCTGCGTTCTTCATCGATGC-3' and ITS3 5'-GCATCGATGAAGAACGCAGC-3'. Then, amplified genomic DNA was sequenced by Macrogen company (Korea). Finally, microorganisms were identified using NCBI blast tool.

2.2. PREPARATION OF BORON SOLUTIONS

SPT, DOT, BA, STD, ZB were kindly provided by the National Boron Research Institute-BOREN (Ankara, Turkey). Stock solutions for DOT, BA and STD were prepared at the concentration of 45 mg/ml and for SPT, the stock solution was prepared at the concentration of 30 mg /ml in Tryptic soy broth (TSB, #CM0129, Thermo Scientific, UK) and Sabouraud dextrose broth (SDB, #108339, Merck Millipore, USA). Because of low solubility properties of zinc borate, it did not dissolve in aqueous solutions. Therefore, antimicrobial effect of ZB was only investigated with modified disc diffusion method.

2.3. PREPARATION OF PLURONIC F68 AND F127 SOLUTIONS

Pluronic F68 (pF68, Carbopol Ultrez-21, Lubrizol, USA) and Pluronic F127 (pF127, Carbopol Ultrez-21, Lubrizol, USA) block copolymers were dissolved two per cent *(w/v)* in the TSB and SDB. Solutions were stored at 4 °C to provide complete dissolution. After dissolution, the solutions were autoclaved.

2.4. STANDARD AND MODIFIED DISC DIFFUSION METHODS

Antimicrobial effects of boron derivatives were determined by two types of disc diffusion methods; NCCLS standard disc diffusion method and modified disc diffusion method. Firstly, NCCLS standard disc diffusion method were applied according to literature [64]. Secondly, NCCLS disc diffusion method was modified to test powder boron derivatives on microorganisms. Briefly, bacteria (*E. coli, S. aureus, P. aeruginosa , A. baumanii, B. cereus*), yeast (*C. glabrata, C. albicans, K. marxianus*) and fungi *(A. niger*) species were spread on TSA and SDA for both methods. For standard disc diffusion method, four types of boron derivatives (DOT, STD, SPT and BA) were dissolved in dH_2O. Then, they were added (20 µl) on blank disc and the disks were put on agars. Disc with dH_2O were used as negative control and ofloxacin (five µg/disc) and nystatin (30 µg/disc) were used as positive controls for bacteria, yeast and fungi, respectively. After microorganisms were incubated at 37 ºC for bacteria for 24 hours, 48 hours for the yeast and 72 hours for fungi, inhibition zones were determined for each microorganism.

For modified disc diffusion method, 20 µl dH_2O added on blank disc for making sticky surface on blank disc. Then disc surface filled with a solid form (~5mg) of boron derivatives (DOT, STD, SPT, ZB and BA) and placed on agars. Disc with dH_2O used as negative control and ofloxacin (five µg/disc) and nystatin (30 µg/disc) were used as positive controls for bacteria, yeast and fungi, respectively. After incubation at 37 ºC for bacteria for 24 hours, 48 hours for yeast and 72 hours for fungi, inhibition zones were determined for each microorganism.

2.5. MINIMUM INHIBITORY CONCENTRATION METHOD

Standard NCCLS method was applied [64] for the determination of MIC for each boron derivatives. Antimicrobial susceptibility testing were carried out using 100 µl of cell suspension containing 10^8 CFU/ml bacteria, 10^6 CFU/ml yeast, 10^4 spore/ml fungi. Then the standard MIC method was applied to microorganism which is summarized in Table 2.1. Antimicrobial effects of all boron derivatives were determined. Then two per cent *(w/v)* pF68 and pF127 and boron derivatives were combined to determine the possible synergistic antimicrobial effect of boron derivatives with pluronics as shown in Table 2.2.

Table 2.1. Preparation of Standard MIC Method for Boron Derivatives

10^{-1} Dilution	100 µl media (TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-2} Dilution	100 µl media (TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-3} Dilution	100 µl media (TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-4} Dilution	100 µl media (TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-5} Dilution	100 µl media (TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution - 100 µl discarded from well
Negative Control	100 µl media (TSB or SDB) + 100 µl microorganism suspension

Table 2.2. Preparation of Standard MIC Method for Boron Derivatives with Pluronic F68 and F127

10^{-1} Dilution	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-2} Dilution	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-3} Dilution	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-4} Dilution	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution
10^{-5} Dilution	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension + 100 µl Boron derivatives solution - 100 µl discarded from well
Negative Control	100 µl media (2% *(w/v)* pluronic F68 and F127 with TSB or SDB) + 100 µl microorganism suspension

2.6. ANTIMICROBIAL DIAPER PREPARATION

After determination of antimicrobial effect of boron derivatives, DOT and pF68 combination was chosen for antimicrobial diaper test. Firstly, commercial adult diaper (Selpak, Turkey) containing SAP was cut into small pieces (four cm x four cm). Diaper pieces were sterilized at 70 °C for overnight. Then, 640 mg of DOT (40 mg DOT for one cm^2 or 640 mg DOT + 320 mg pF68 combinations were added to absorbent polymer side of the each diaper. All absorbent polymer top side surface was covered by DOT or DOT + pF68 combinations. 25 ml of respective media (TSB and SDB) containing, 10^8 CFU/ml bacteria, 10^6 CFU/ml yeast, and 10^4 spore/ml fungi was poured to the top sheets of the

diapers including DOT, DOT + pF68 combinations or negative control. Then, diapers were incubated at 37 ºC for bacteria 24 hours, 48 hours for the yeast and 72 hours for fungi species. After respective incubation periods, all diapers were put in the sterile Erlenmeyer flasks containing phosphate buffered saline (PBS, 200 ml) and were shaken for 20 min at 150 rpm to homogenize diapers in PBS. One ml of sample was taken from all Erlenmeyer flasks in triplicate and serial dilutions (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5}) were made with sterile PBS. Samples (100µl) were taken from every dilution and spread on TSA and SDA agars for all microorganisms. After petri dishes were incubated at 37 ºC for bacteria 24 hours, 48 hours for yeast and 72 hours for fungi, antimicrobial activity efficiencies and percentages were determined with counting colonies on agar.

3. RESULTS

3.1. IDENTIFICATION OF MICROORGANISMS

MIS method was applied to pathogenic microorganism for identification. Five different types of bacteria were identified by Sherlock library match system which are (Appendix A.1, 2, 3, 4, 5) *B. cereus, E. coli, P. aureginosa, A. baumanii, S. aureus.*

3.1.1. rDNA Based Sequencing for Identification of Yeast and Fungi

Yeast and fungal microorganisms were identified with rDNA based sequencing. Five different types of microorganism's genomic DNA were isolated as described previously. Isolated genomic DNAs are sequenced by Macrogen Company and sequences were compared with NCBI search tool for identification microorganism. *C. glabrata, C. albicans, K. marxianus, A. niger* were identified by NCBI alignment tool (Data not shown).

3.2. ANTIMICROBIAL EFFECTS OF BORON DERIVATIVES DETERMINATION BY DISK DIFFUSSION METHOD

Antimicrobial effects of boron derivatives (DOT, STD, SPT, ZB, and BA) against selected microorganisms (*E. coli, S. aureus, P. aureginosa, A. baumanii, B. cereus, C. glabrata, C. albicans, K. marxianus, A. niger*) were evaluated using standard disc diffusion method. The results revealed that only DOT inhibited growth of *B. cereus* and showed 10 mm inhibition zone (Figure 3.1).

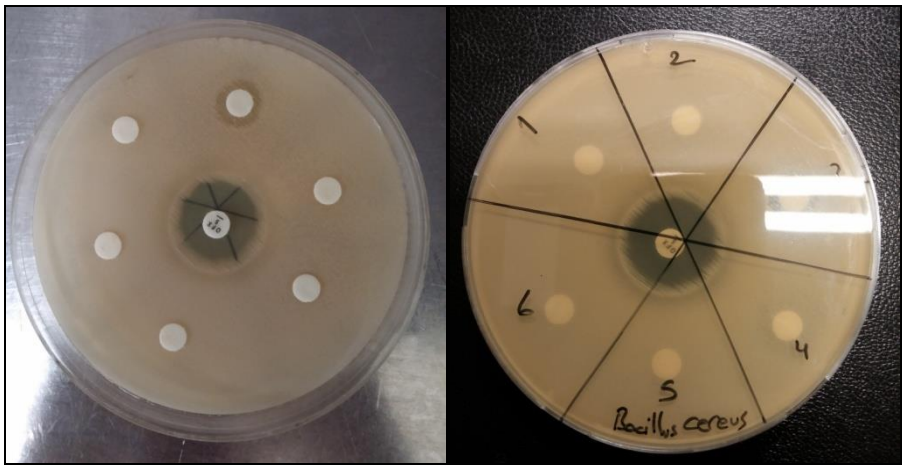

Figure 3.1. Antimicrobial effects of various boron derivatives against *Bacillus cereus*. Each number represents a different type of boron derivatives, pF68 and pF 127 (1. BA, 2. SPT, 3.DOT, 4.STD, 5. pF68, 6. pF127). Positive control: Ofloxacin

3.2.1. Modified Disk Diffusion Method for Analysis Antimicrobial Effects of Boron Derivatives

Modified disc diffusion method was carried out on selected microorganisms (*E. coli*, *S. aureus*, *P. aureginosa*, *A. baumanii*, *B. cereus*, *C. glabrata*, *C. albicans*, *K. marxianus*, *A. niger*). Five different boron derivatives' (BA, SPT, DOT, STD and ZB) antimicrobial effects were investigated using modified dick diffusion method. Results showed that all boron derivatives exhibited different antimicrobial effect against microorganisms tested. In general, ZB exhibited the lowest effect against microorganisms compared to other boron derivates. The data suggested that all boron derivatives showed more inhibitory effects against yeast and fungi with respect to bacteria. When BA exposed to *P. aureginosa*, *A. baumanii*, *B. cereus*, *E. coli and S. aureus* for 24 h, inhibition zones were found to be 23, 13, 17 and seven mm, respectively. Diameter of inhibition zones around SPT impregnated discs were found as 12, 11 15 and 13 mm on *P. aureginosa*, *A. baumanii*, *B. cereus*, *E. coli and S. aureus* inoculated plates, respectively. Moreover, when bacteria were treated with DOT containing discs, inhibition zones were observed to be 18, 14, 18 and seven mm, respectively. As bacteria exposed to STD containing discs, there was not any inhibition zone on *P. aureginosa* inoculated plates but for *A. baumanii*, *B. cereus* and *E. coli*, inhibition zones were found to be eight, 15 and 13 mm, respectively. Lastly, as the powder

form of ZB exposed to bacteria, there were no significant antimicrobial effect. As a result, data suggested that DOT was the most effective boron derivatives against bacteria compared to other types of boron derivatives (Figure 3.2, 3.3, 3.4) (Table 3.1). In addition, results revealed that *E. coli* was the most resistant bacteria strain against all boron derivatives.

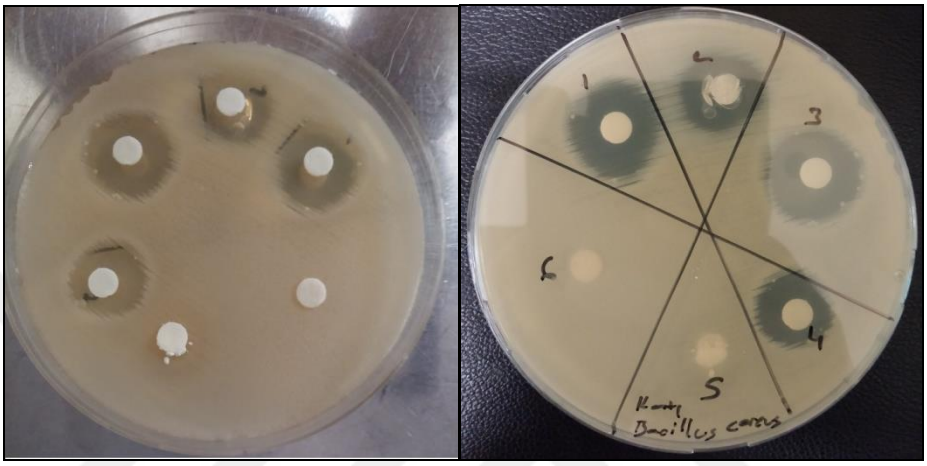

Figure 3.2. Antimicrobial effects of various boron derivatives against *Bacillus cereus*. Each number represents a different type of boron derivatives, pF68 and pF 127 (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

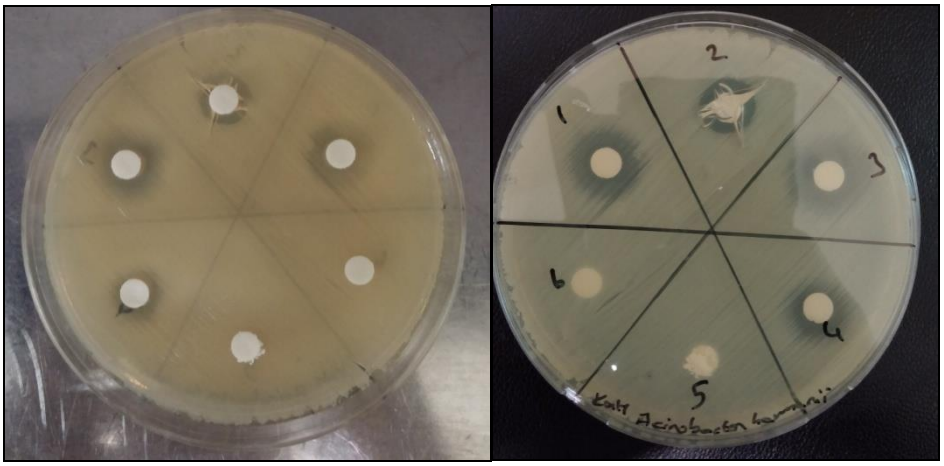

Figure 3.3. Antimicrobial effects of various boron derivatives against *A. baumannii*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

Figure 3.4. Antimicrobial effects of various boron derivatives against *E. coli*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

Secondly, boron derivatives were tested against yeast (*C. albicans, K. marxianus, C. glabrata*) and fungal species (*A. niger*). Diameter of inhibition zones around the boron derivatives impregnated discs determined after modified disc diffusion method performed and the plates were incubated at 37 ºC, 48 h for yeast and fungi for 72 h. According to the results, diameter of inhibition zones of BA on *C. albicans, K. marxianus* and *A. niger* were determined as 40, 23 and 30 mm. However, there was no inhibitory activity of BA against *C. glabrata*. When SPT exposed to microorganisms (*C. albicans, K. marxianus, C. glabrata and A. niger*), inhibition zones were found to be 34, 25, 13 and 29 mm, respectively. Antifungal activity of another boron derivative, DOT, against yeast and fungi were also investigated, and inhibition zones were calculated as 31, nine, 27 and 26 mm, respectively. When microorganisms were exposed to STD, inhibition zones were found as 30, eight, 10 and 24 mm, respectively. Lastly, treatment of ZB only affected *C. albicans* and *A. niger* in which inhibition zones were found to be 11 mm for both microorganism but there were no antimicrobial against *C. glabrata*. As a conclusion, boron derivatives displayed remarkable growth inhibition in yeast and fungi (Table 4). Especially, DOT was detected to be the most effective boron derivative to inhibit growth of microorganism (Figure 3.5, 3.6, 3.7, 3.8). Additionally, *C. glabrata* was found to be the most resistance strain against boron derivatives, even some boron derivative was remained ineffective against *C. glabrata*.

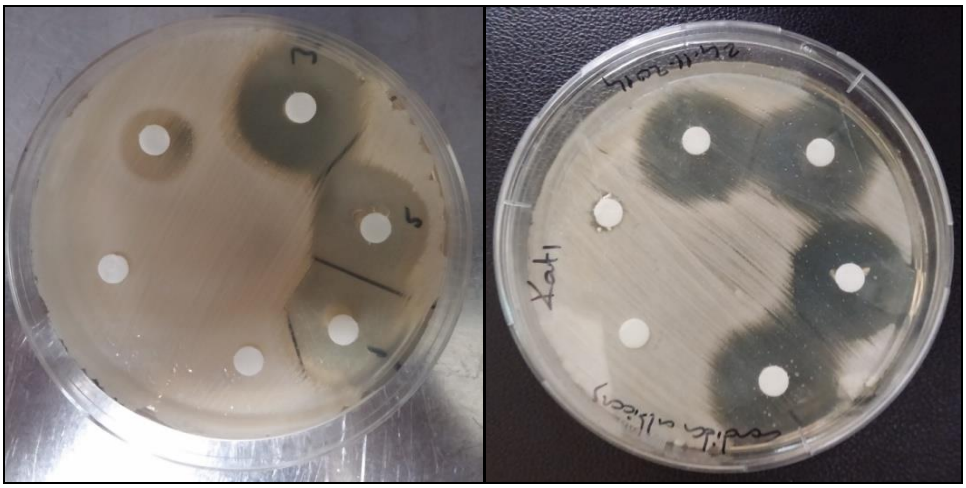

Figure 3.5. Antimicrobial effects of various boron derivatives against *C. albicans*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

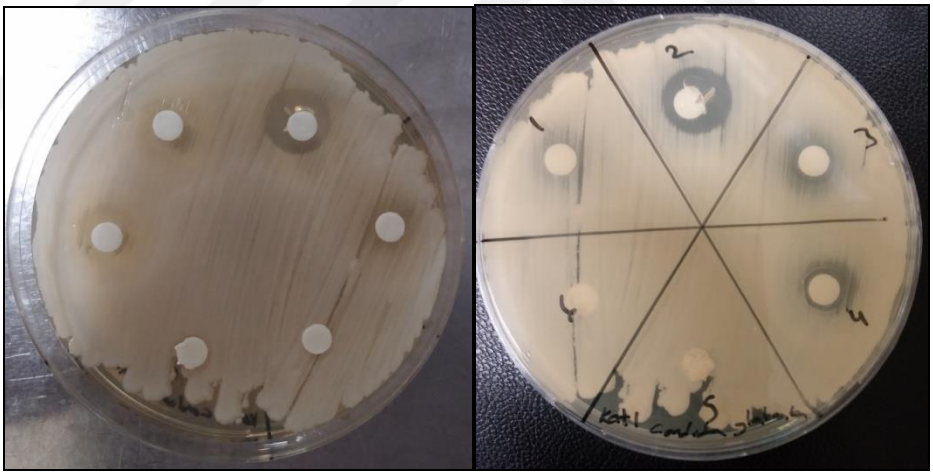

Figure 3.6. Antimicrobial effects of various boron derivatives against *C. glabrata*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

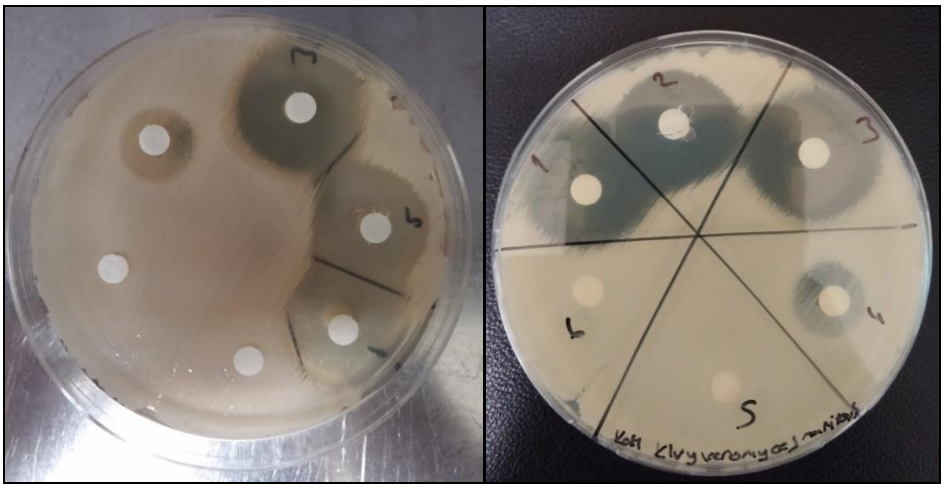

Figure 3.7. Antimicrobial effects of various boron derivatives against *K. marxianus*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

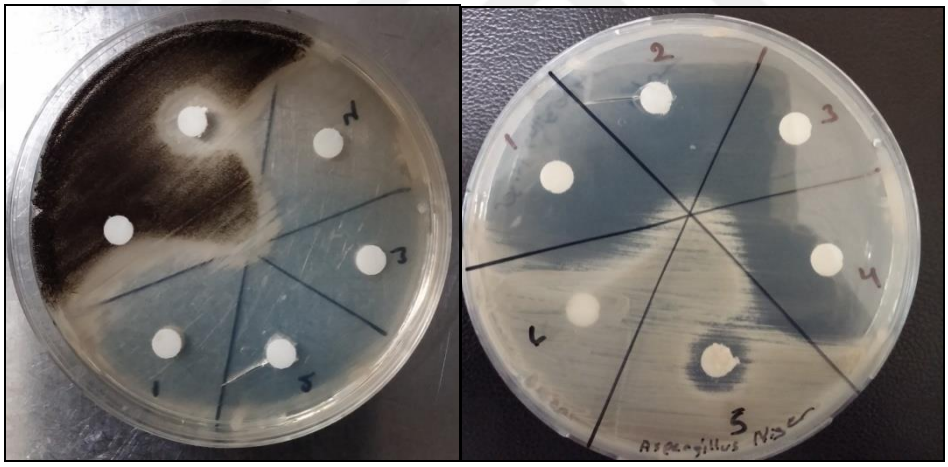

Figure 3.8. Antimicrobial effects of various boron derivatives against *A. niger*. Each number represents a different type of boron derivatives, (1. BA, 2. SPT, 3.DOT, 4.STD, 5. ZB, 6. NC).

Table 3.1. Modified disc diffusion method for an antimicrobial susceptibility test performed on microorganisms. Inhibition zones were determined for all boron derivatives.

Boron Derivatives	***S. aureus***	***P. aureginosa***	***A. baumannii***	***B. cereus***	**E. coli**	***K. marxianus***	***A. niger***	***C. glabrata***	***C. albicans***
BA	0 mm	23 mm	13 mm	17 mm	7 mm	23 mm	40 mm	0 mm	30 mm
SPT	16 mm	12 mm	11 mm	15 mm	13 mm	25 mm	34 mm	13 mm	29 mm
DOT	21 mm	18 mm	14 mm	18 mm	7 mm	27 mm	31 mm	9 mm	26 mm
STD	16 mm	0 mm	8 mm	15 mm	13 mm	10 mm	30 mm	8 mm	24 mm
ZB	13 mm	0 mm	0 mm	0 mm	0 mm	0 mm	11 mm	0 mm	11 mm
NC	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm

3.3. ANTIMICROBIAL EFFECT OF AQUAOUS SOLUTIONS OF BORON DERIVATIVES DETERMİNED BY MINIMUM INHIBITORY CONCENTRATION (MIC) ANALYSIS

MIC concentrations for four boron derivatives (BA, SPT, DOT and STD) aqueous solutions were determined by broth dilution methods as previously described and shown on Table 2.1. MICs of pF68 and pF127 were also tested to observe potential antimicrobial effects of poloxamers. The result indicates that pF68 and pF127 exposure to microorganism alone did not significantly affected the microbial growth. Similar to disc diffusion assay's results, all boron derivatives showed antimicrobial effects. For BA solution, MICs were found as ranging from 1125 to 532.5 µg/ml. When cells exposed to SPT solution, even lower concentrations was found to be effective against *B. cereus* (70.31 µg/ml). SPT also displayed inhibitory effects against yeast cells (*C. albicans, K. marxianus*, *C. glabrata*), and MIC values were ranging from 140.25 µg/ml to 562.5 µg/ml. Although, SPT was more effective than BA against bacteria and yeast species tested, it was not affective against *A. niger* compared to BA. DOT treatment exhibited similar

antimicrobial effects against bacterial and fungal strains and MIC values were also similar to BA and SPT, but DOT showed significant anticandidal effect against yeast cells especially against *C. albicans* strains. Lastly, STD was tested against microorganisms and found to effective against bacterial species but it did not show any growth inhibitory effect against yeast and fungal strains compared to other boron compounds. To sum up, DOT was found to be the most effective boron derivative against microorganisms tested (Table 3.2).

Table 3.2 MICs for four boron derivatives and pluronics were determined by the broth dilution method.

Boron Derivatives (µg/ml)	***S. aureus***	***P. aureginosa***	***A. baumannii***	***B. cereus***	***E. coli***	***K. marxianus***	***C. albicans***	***C. glabrata***	***A. niger***
BA	>1125	1125	562.5	562.5	>1125	140.625	140.625	281.25	281.25
SPT	1125	1125	562.5	70,31	281.25	281.25	140.625	562.5	140.625
DOT	1125	1125	281.25	281.25	562.5	140.625	70,31	281.25	140.625
STD	750	750	375	375	375	93.75	93.75	187.5	187.5
Pluronic F68	>50000	>50,000	>50,000	>50,000	>50,000	>50,000	>50,000	>50,000	>50,000
Pluronic F127	>50000	>50000	>50,000	>50000	>50000	>50000	>50000	>50000	>50000

3.3.1. MICs for Pluronics and Boron Derivative Combinations

After determination of MIC values for boron chemicals with NCLLS method [64], antimicrobial effects of pluronics-boron derivative combinations were investigated. pF68 and pF127 block copolymers were prepared as two per cent (w/v) aqueous solution in respective media and different concentrations (11.25, 5.62, 2.81, 1.4, 0.7 and 0.3 mg/ml) of borates combined with pluronics. Then, potential synergetic antimicrobial activity of combinations were examined against microorganisms. The results revealed that all boron derivatives-pluronics combination has unique antimicrobial effects against each microorganisms. Inhibition of growth percentage for all boron derivatives-pF68 and -pF127 combinations were calculated for boron derivatives' 1/2 x MIC values.

3.3.2. Antimicrobial Susceptibility Test for Boric Acid, Boric Acid + Pluronic F68 and Pluronic F127 Combinations

Firstly, antimicrobial effects of BA and pluronics were examined against bacterial strains (*A. baumanii, E. coli, S. aureus, P. aeruginosa B. cereus*). Percentage of inhibition of growth BA alone (1/2 x MIC) was found to be 74 per cent against *A. baumanii*. Moreover, BA-pF68 and BA-pF127 combinations increased the antimicrobial effect as 15 per cent and 6.89 per cent, respectively (Figure 3.9). Percentage of growth inhibition BA alone was found to be 51 per cent on *E. coli* and pluronic combinations raised antibacterial effect of BA as 17 per cent and 16 per cent , respectively (Figure 3.10). BA alone percentage of inhibition of growth on *S. aureus* examined as 29.35 per cent and pluronic combinations increased antimicrobial effect as 18 per cent and four per cent , respectively (Figure 3.11). When BA and pluronic combinations expose to *P.aeruginosa* strain, BA alone showed 47.5 per cent inhibition and pluronic combinations increased antimicrobial effect as 17.77 per cent and 38.41 per cent , respectively (Figure 3.12). Lastly, antibacterial effect of BA and pluronics percentage of inhibitions of growth were determined on *B. cereus* strain. results showed that percentage of inhibition of growth found to be 15.83 per cent and BA-pluronic combinations increased the antimicrobial effect as 16 per cent and 21 per cent, respectively (Figure 3.13).

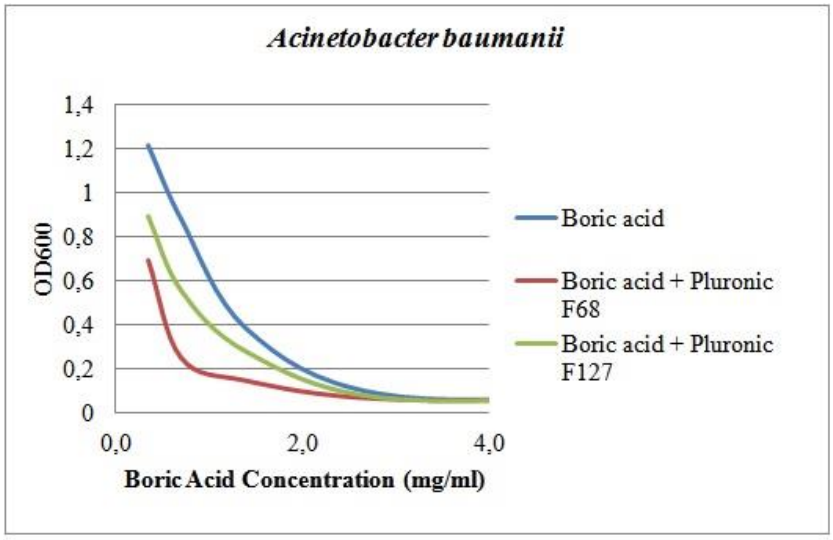

Figure 3.9. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *A. baumannii*. Negative control (Tryptic soy broth)

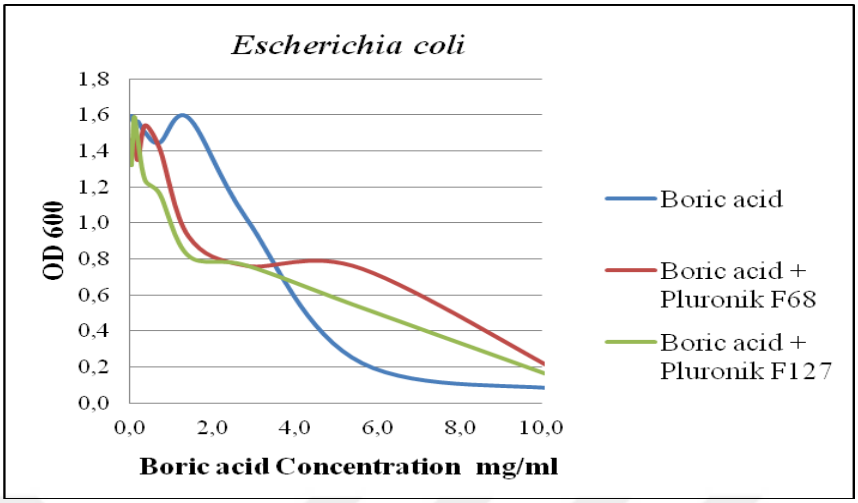

Figure 3.10. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *E. coli*. Negative control (Tryptic soy broth)

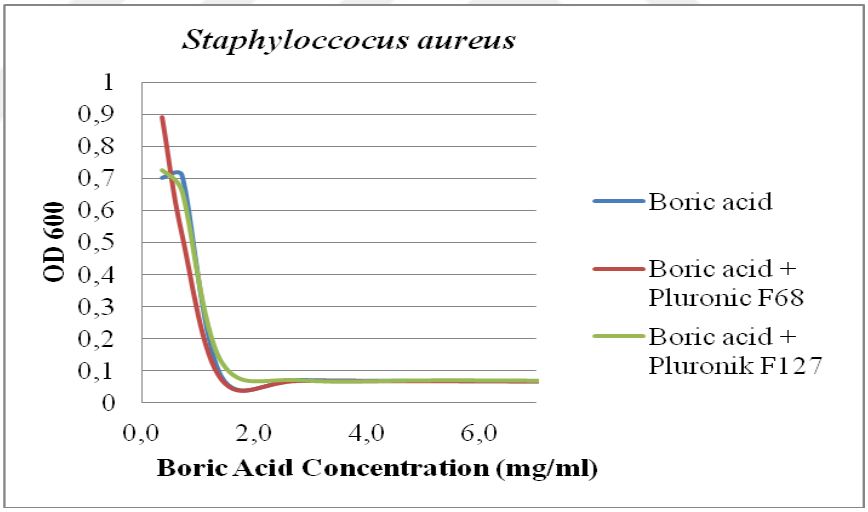

Figure 3.11. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *S.aureus*. Negative control (Tryptic soy broth)

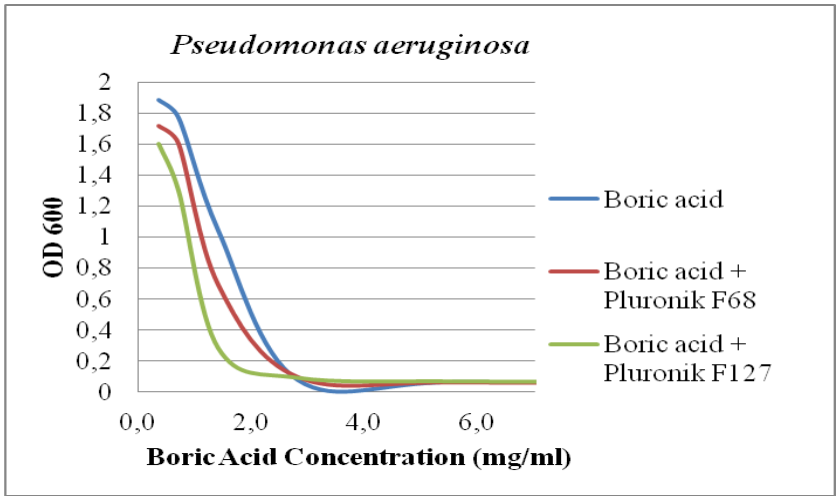

Figure 3.12. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *P. aeruginosa*. Negative control (Tryptic soy broth)

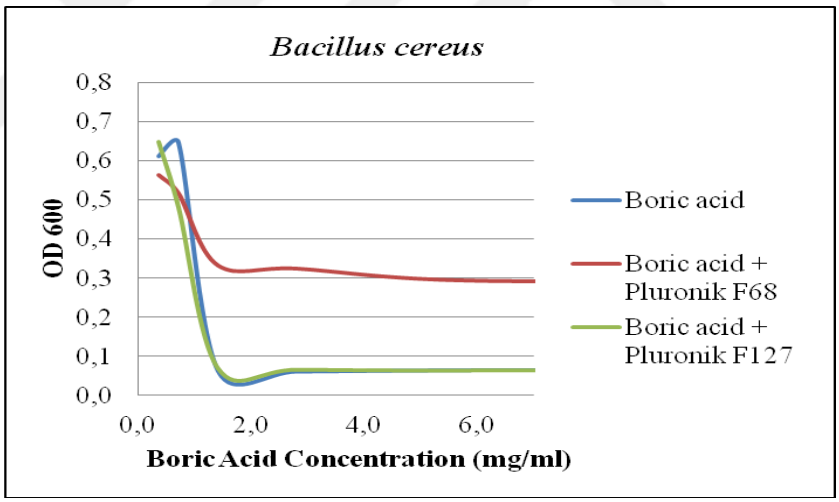

Figure 3.13. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *B. cereus*. Negative control (Tryptic soy broth)

BA and pluronics combinations antimicrobial effects also investigated on yeast (*C. albicans, K. marxianus* and *C. glabrata*) and fungi (*A. niger*) strains. BA treatment on *C. glabrata* percentage of inhibition growth found to be 40.6 per cent, BA with pF68 and pF127 combinations increased antimicrobial activity as 63.7 per cent and 65.26 per cent , respectively (Figure 3.14). Another *Candida* species that *C. albicans* exposed to 1.406 mg/ml BA (1/2 x MIC) there were not any anticandidal activity observed but with pF68

and pF127 combination increased antimicrobial effect as 39.9 per cent and 22.4 per cent, respectively (Figure 3.15). For another yeast strain *K. marxianus*, BA (1/2 x MIC) were not affected on cells, with pF68 and pF127 combination contribute to BA percentage of inhibition of cell growth 90 per cent and 6.5 per cent, respectively. BA+pF68 combination were the most effective combination on *K. marxianus* compare to other boron derivative-pluronic combinations antimicrobial effects (Figure 3.16). Finally, for investigation antifungal effect of BA, *A. niger* were used for this experiment. BA antimicrobial effect on fungal cells found as 84.68 per cent and with pluronic combinations only BA+pF68 combination increased antimicrobial activity (10.52 per cent) of BA (Figure 3.17). As a conclusion, when cells were treated BA with 1/2 x MIC values for each microorganisms, the most significant antimicrobial effect observed on *C. glabrata* strain. Moreover, BA and pluronics combinations antimicrobial synergic effect results showed that BA+pF68 mixed solution antimicrobial effect peaked on *K. marxianus* strain. Additionally, antimicrobial effect of BA+pF127 combination spiked on *C. glabrata* strain (Table 3.3).

Table 3.3. Percentages of growth inhibition for Boric acid (BA) and Pluronic F68, F127 treated microorganisms

Microorganism	**NC**	**BA**	**F68+BA**	**F127+BA**
A.baumanii	0,00	74,50	90,03	81,39
P. aeruginosa	0,00	47,51	65,28	85,92
B. cereus	0,00	15,83	32,57	37,40
S.aureus	0,00	29,35	47,48	34,13
E.coli	0,00	34,28	51,23	51,04
C. albicans	0,00	0,00	39,98	22,47
C. glabrata	0,00	40,64	81,94	83,50
A. niger	0,00	84,68	95,20	82,54
K.marxianus	0,00	0,00	90,96	6,52

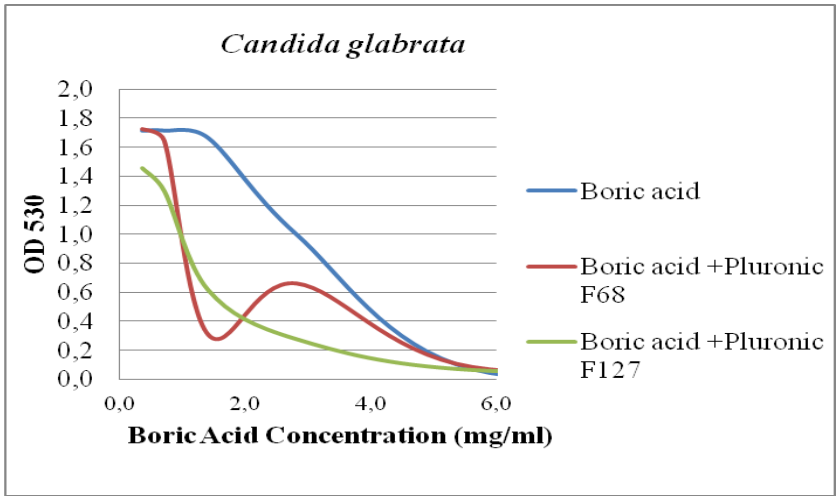

Figure 3.14. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *C. glabrata*. Negative control (Sabouraud dextrose broth)

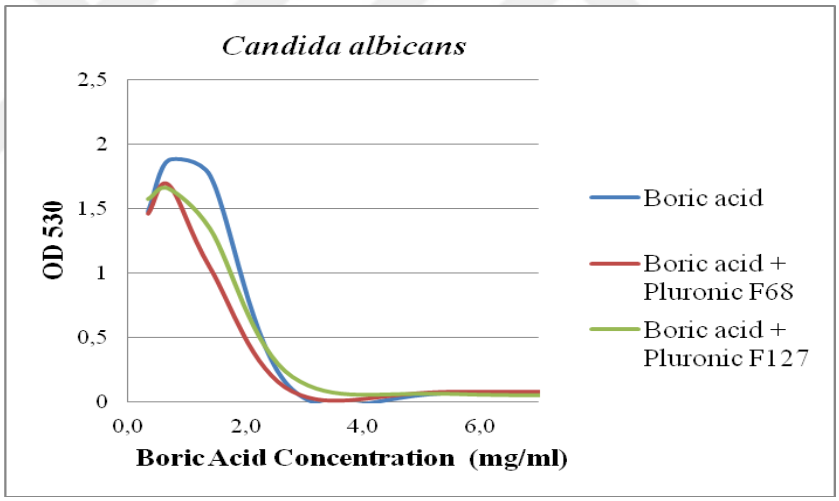

Figure 3.15. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *C. albicans*. Negative control (Sabouraud dextrose broth)

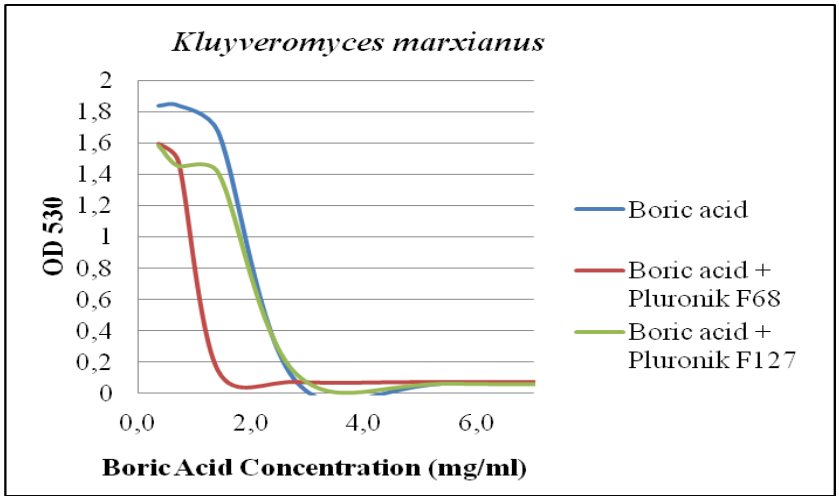

Figure 3.16. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *K. marxianus*. Negative control (Sabouraud dextrose broth)

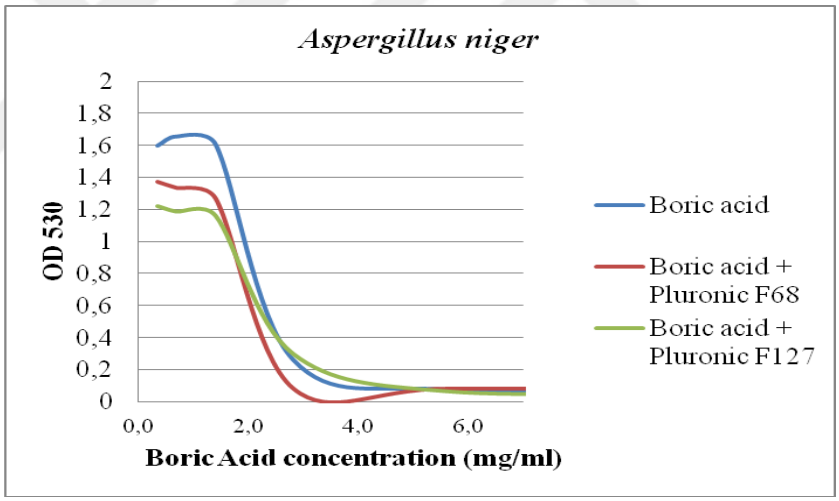

Figure 3.17. Antimicrobial activity of Boric acid and Boric acid - Pluronic F68 and F127 combinations against *A. niger*. Negative control (Sabouraud dextrose broth)

3.3.3. Sodium Pentaborate Tetrahydrate, Sodium Pentaborate Tetrahydrate + Pluronic F68 and Pluronic F127 Combinations Antimicrobial Susceptibility Test

SPT has low solubility properties so aqueous solution of SPT prepared as 30 mg/ml and inhibition percentage calculations conducted with 1/2 x MIC concentration. SPT antibacterial effects were investigated on bacteria strains. Percentage of inhibition of

growth on bacteria strains ,*A. baumannii*, *B. cereus*, *E. coli*, *P.aeruginosa*, *S. aureus*, found to be 37.76 per cent , 24.20 per cent , 25.60 per cent ,24.9 per cent and 13.6 per cent , respectively. pF68 and pF127 combinations with SPT increased antimicrobial effect percentage on microorganisms as 56.79 per cent and 25.76 per cent on *A. baumanii* (Figure 3.18), only pF68 increased SPT inhibition effect 28,62 per cent on *B. cereus* (Figure 3.19), 54.09 per cent and 25.51 per cent on *E. coli* (Figure 3.20), 39.49 per cent and 60.25 per cent on *P. aeruginosa* (Figure 3.21). Pluronics could not increase SPT percentage of inhibition of growth on *S. aureus* (Figure 3.22). We also investigated percentage of inhibition of growth on yeast (*C. albicans*, *C. glabrata* and *K. marxianus*) and fungi (*A. niger*) species. The results revealed that the percentage of inhibition of growth 80 per cent on *C. glabrata* and for *K. marxianus* 60 per cent inhibition observed but there is no inhibition observed on *C. albicans* and *A. niger* strains. pF68 and pF127 combinations with SPT increased antimicrobial effects as 12.15 per cent and 21.79 per cent increased inhibition for *C. albicans* (Figure 3.23), for *C. glabrata* pF68 cannot contribute to SPT to increase inhibition of growth but SPT+pF127 combination increased inhibition 5 per cent (Figure 3.24), for *K. marxianus* combination cannot increase percentage of inhibition of growth (Figure 3.25), for A. niger pF68 and pF127 were ineffective for inhibition contribution to SPT (Figure 3.26) (Table 3.4).

Table 3.4. Percentages of growth inhibition for Sodium pentaborate tetrahydrate (SPT) and Pluronic F68, F127 treated microorganisms

Microorganism	**NC**	**SPT**	**F68+SPT**	**F127+SPT**
A. baumanii	0,00	36,76	63,97	64,10
P. aeruginosa	0,00	24,91	43,37	74,86
B. cereus	0,00	24,20	51,90	22,11
S. aureus	0,00	13,65	24,87	29,42
E. coli	0,00	25,60	56,00	42,102
C. albicans	0,00	0,00	0,00	13,96
C. glabrata	0,00	80,86	79,54	85,87
A. niger	0,00	81,54	77,13	82,85
K. marxianus	0,00	83,11	81,65	85,40

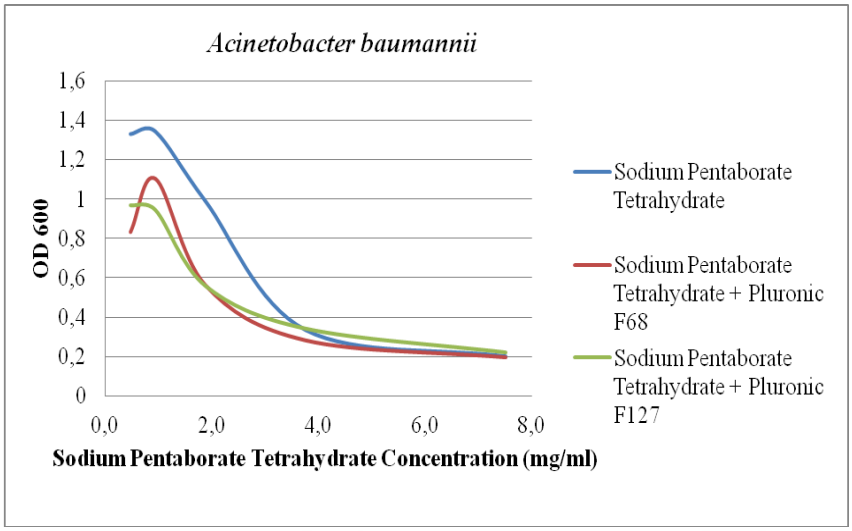

Figure 3.18. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *A. baumannii*. Negative control (Tryptic soy broth)

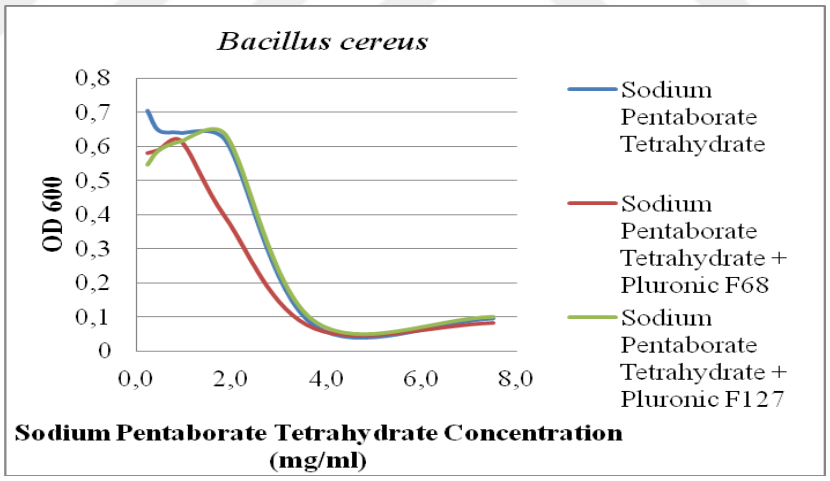

Figure 3.19.. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *B. cereus*. Negative control (Tryptic soy broth)

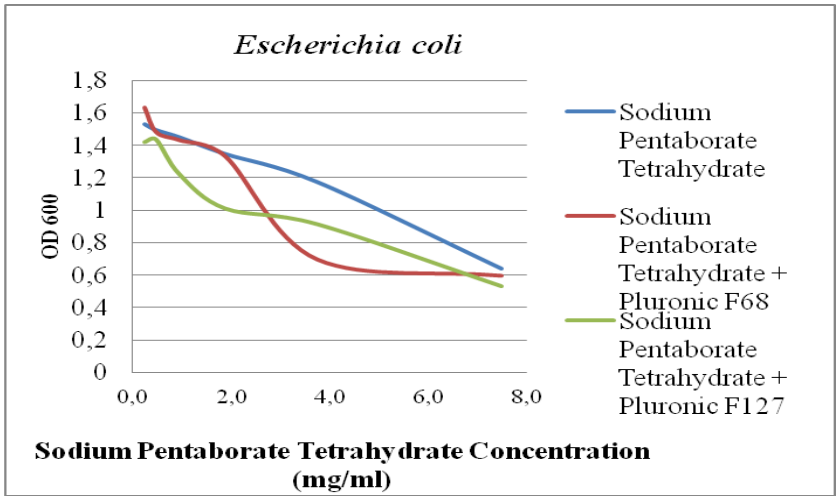

Figure 3.20. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *E. coli*. Negative control (Tryptic soy broth)

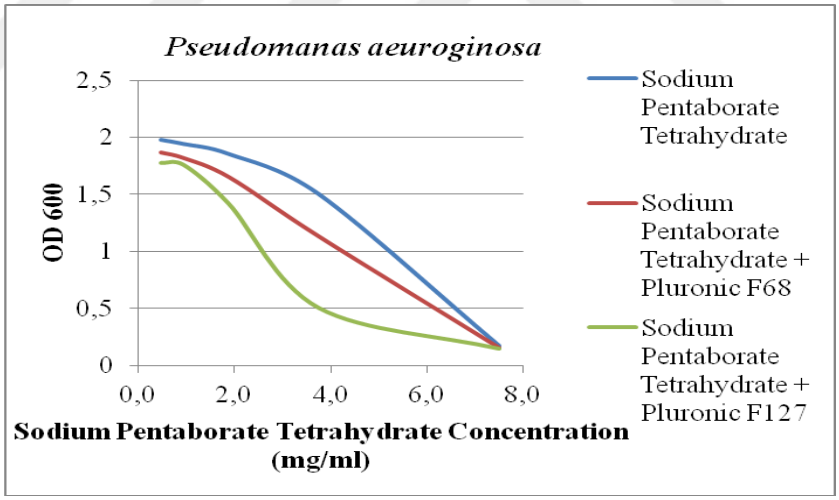

Figure 3.21. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *P.aeruginosa*. Negative control (Tryptic soy broth)

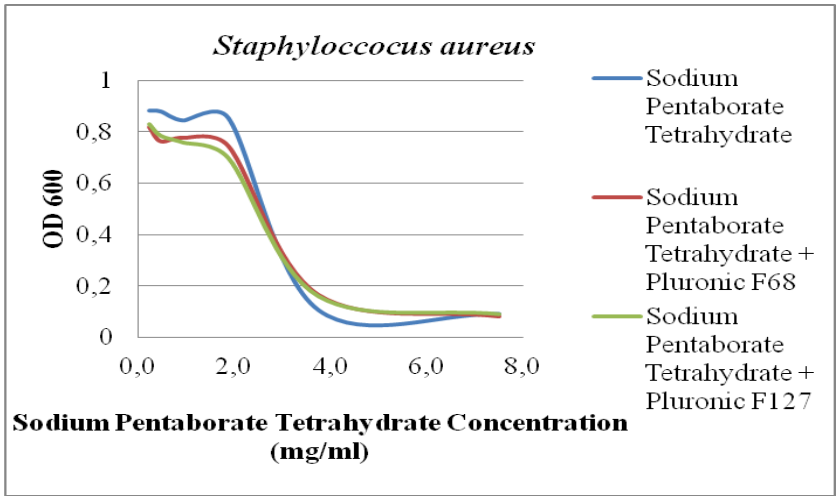

Figure 3.22. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *S. aureus*. Negative control (Tryptic soy broth)

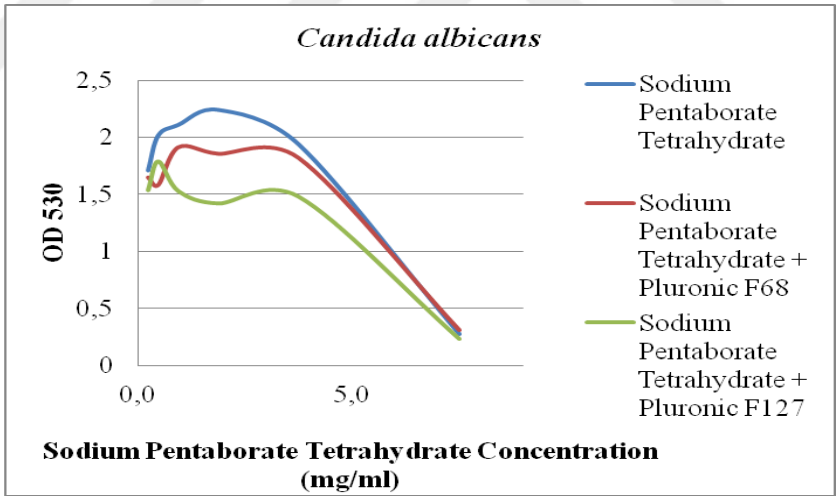

Figure 3.23. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *C. albicans*. Negative control (Sabouraud dextrose broth)

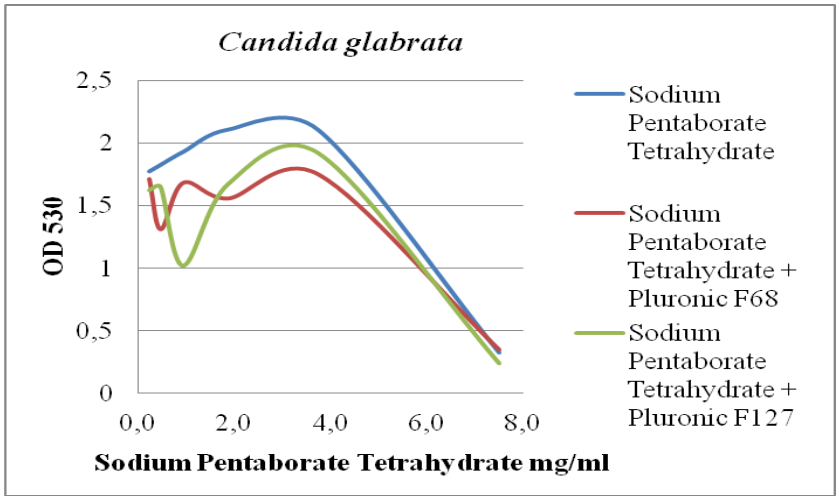

Figure 3.24. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *C. glabrata*. Negative control (Sabouraud dextrose broth)

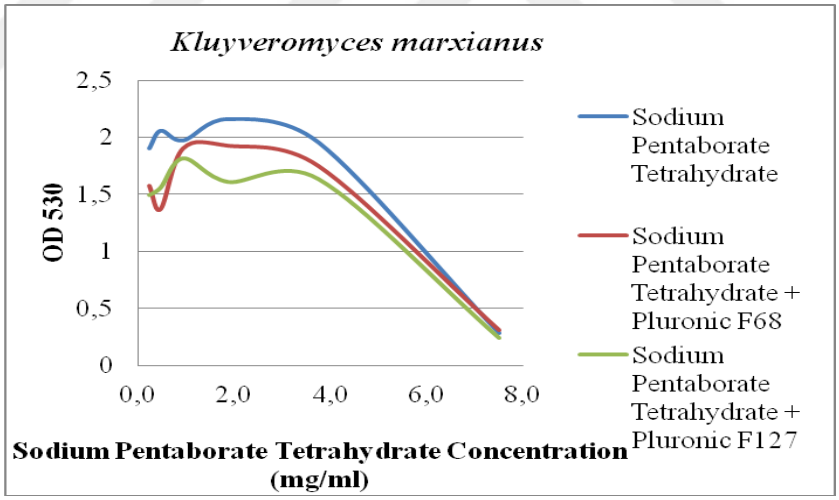

Figure 3.25. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *K. marxianus*. Negative control (Sabouraud dextrose broth)

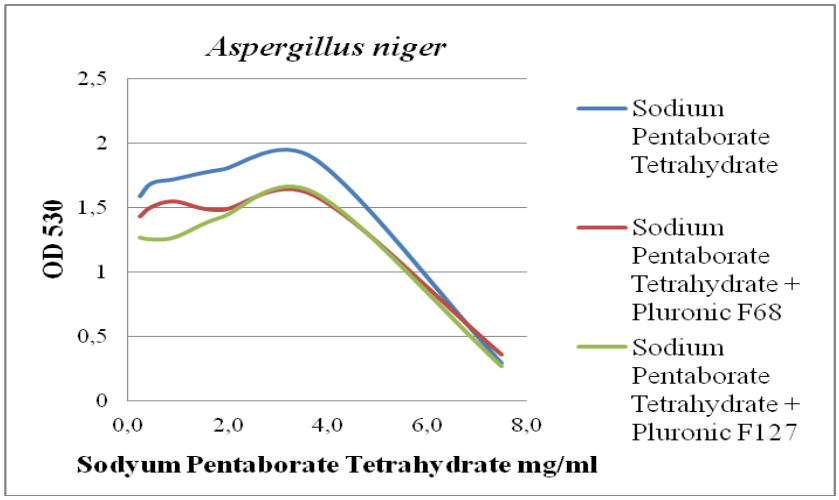

Figure 3.26. Antimicrobial activity of Sodium pentaborate tetrahydrate and Sodium pentaborate tetrahydrate- pF68 and pF127 against *A. niger*. Negative control (Sabouraud dextrose broth)

3.3.4. Sodium Tetraborate Decahdyrate, Sodium Tetraborate Decahdyrate+ Pluronic F68 and F127 Combinations Antimicrobial Analysis

STD is another boron derivative which being used as a biocide agent in the industry. Therefore, antimicrobial susceptibility experiment performed with STD. When 1/2 x MIC SPT exposure to bacteria strains, SPT inhibited *A. baumanii*, *E. coli*, *S. aureus*, *P. aeruginosa* and *B. cereus* growth to 86.00 per cent , 49.15 per cent , 85.24 per cent , 57.60 per cent and 87.87 per cent , respectively (Figure 3.27, 3.28, 3.29, 3.30, 3.31). Combination of SPT with pF68 and pF127 couldn't increase antibacterial activity of SPT on bacteria strains except *S. aureus* which combination with pF68 and pF127 increased antimicrobial effect as 41.65 per cent and 19.52 per cent, respectively. When we focused on SPT inhibition effect on yeast and fungi, results showed that SPT exhibit 49.67 per cent inhibition on *C. albicans* (Figure 3.32), 40.42 per cent inhibition on *C. glabrata* (Figure 3.33), 60.31 per cent inhibition on *K. marxianus* (Figure 3.34) and 43.56 per cent inhibition on *A. niger*. When pF68 and pF127- SPT synergic effect investigated inhibition of growth increase to 16.54 per cent and 55.24 per cent for *C. albicans*, 57.71 per cent and 64.50 per cent for *C. glabrata*, there was no inhibition of growth increase obtained for pF68-SPT

combination and 15 per cent for pF127-SPT combination, for *K. marxianus* 51.35 per cent and 32.54 per cent for *A. niger* (Figure 3.35) inhibition of growth increase observed. To sum up the results SPT was found highly effective on bacteria strains, but it is not effective on yeast and fungi cells compare to bacteria species. Also SPT-pluronics combinations could not increase inhibition of growth for bacteria, yeast and fungi strains compare to another boron derivative-pluronic combinations (Table 3.5).

Table 3.5. Percentages of growth inhibition for Sodium tetraborate decahydrate (STD) and Pluronic F68, F127 treated microorganisms

Microorganism	**NC**	**STD**	**F68+STD**	**F127+STD**
A. baumanii	0,00	86,00	85,68	79,38
P. aeruginosa	0,00	85,24	65,21	81,14
B. cereus	0,00	87,87	88,35	88,11
S. aureus	0,00	49,15	41,65	20,01
E. coli	0,00	47,72	51,42	40,00
C. albicans	0,00	49,67	49,67	74,50
C. glabrata	0,00	40,43	65,22	81,15
A. niger	0,00	43,57	66,27	70,97
K. marxianus	0,00	60,61	53,84	76,41

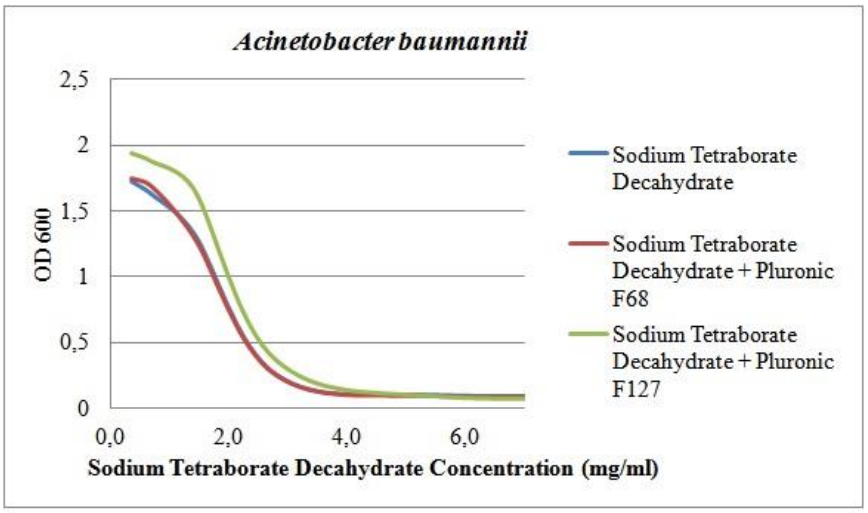

Figure 3.27. Antimicrobial activity of tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *A. baumannii*. Negative control (Tryptic soy broth)

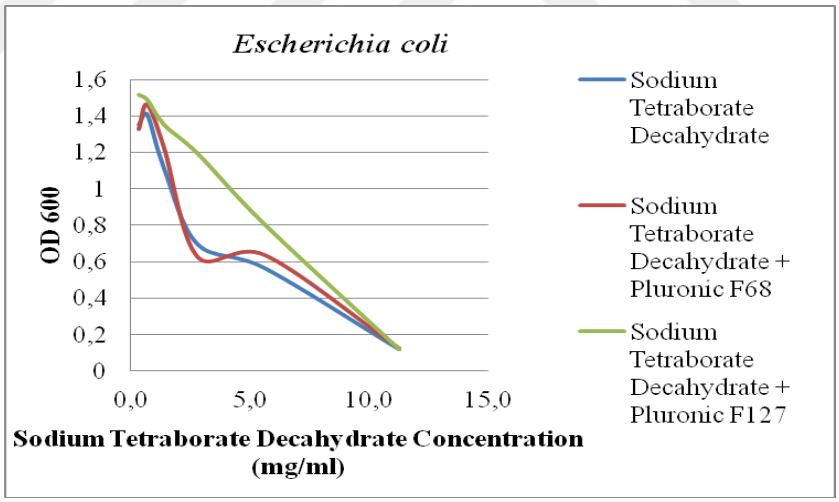

Figure 3.28. Antimicrobial activity of tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *E. coli*. Negative control (Tryptic soy broth)

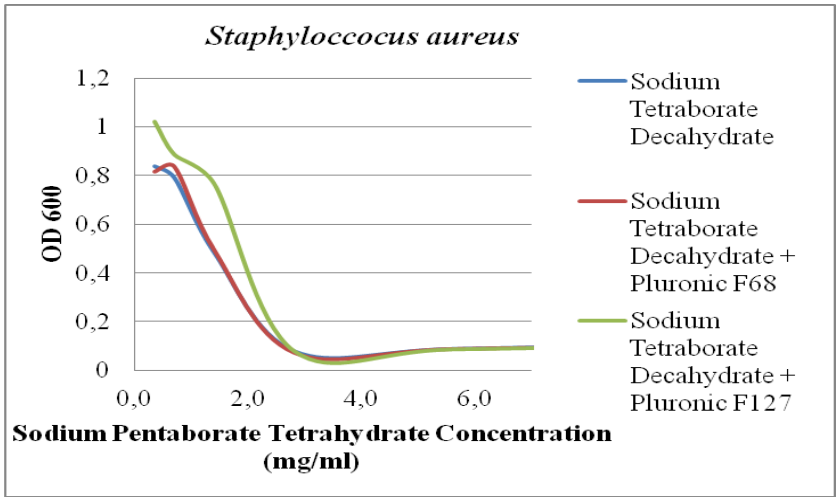

Figure 3.29. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *S. aureus*. Negative control (Tryptic soy broth)

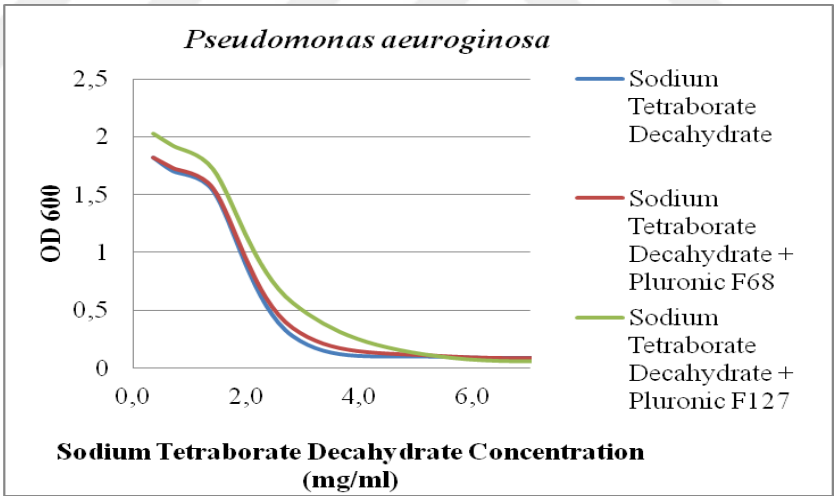

Figure 3.30. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *P. aeruginosa*. Negative control (Tryptic soy broth)

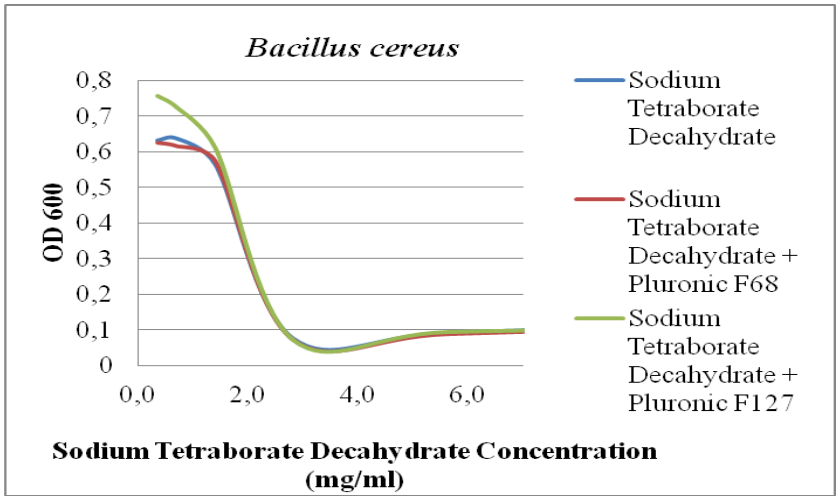

Figure 3.31. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *B. cereus*. Negative control (Tryptic soy broth)

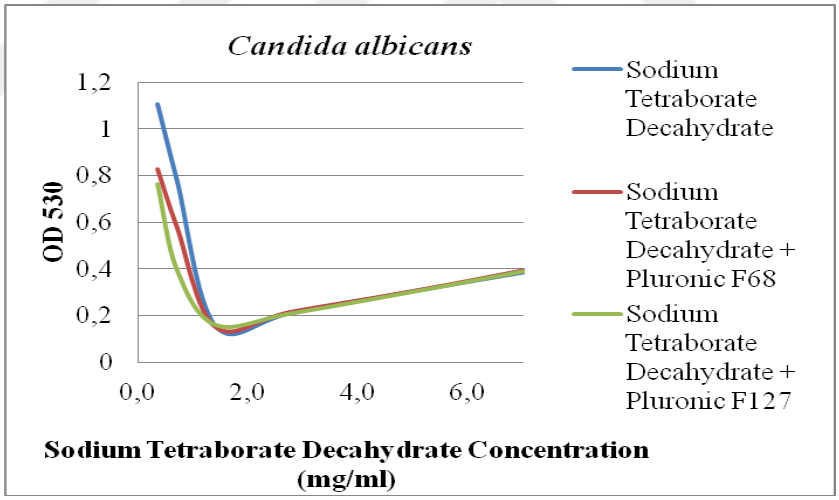

Figure 3.32. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *C. albicans*. Negative control (Sabouraud dextrose broth)

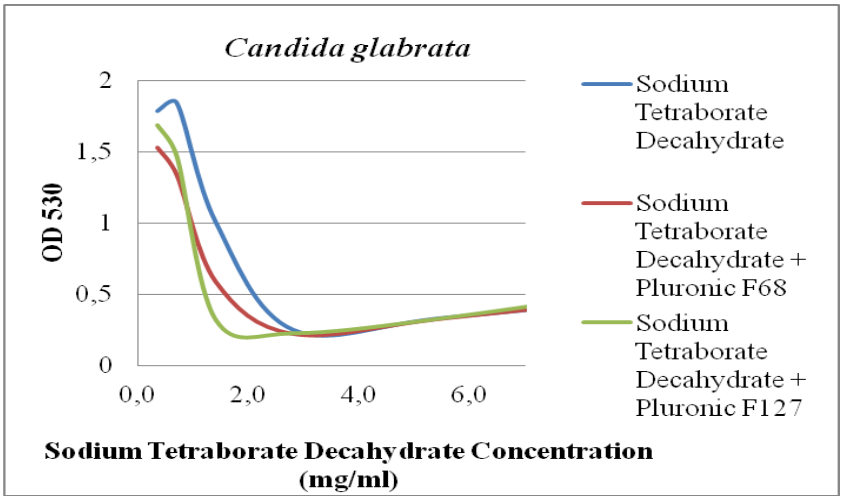

Figure 3.33. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *C. glabrata*. Negative control (Sabouraud dextrose broth)

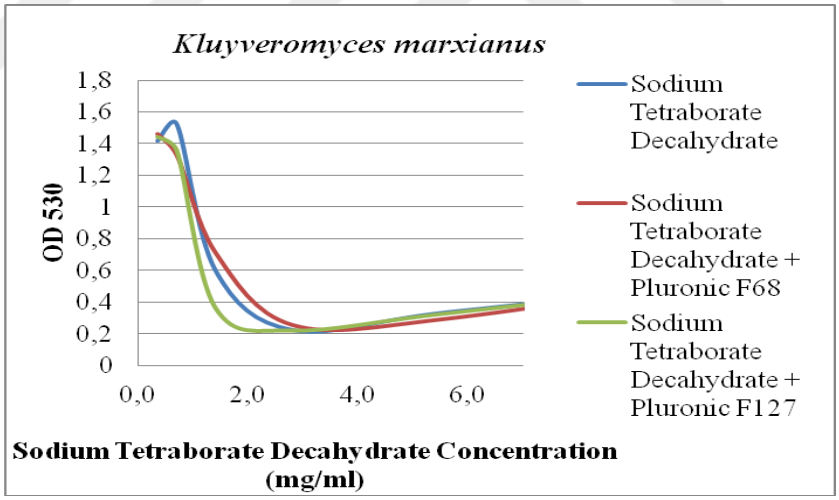

Figure 3.34. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *K. marxianus*. Negative control (Sabouraud dextrose broth)

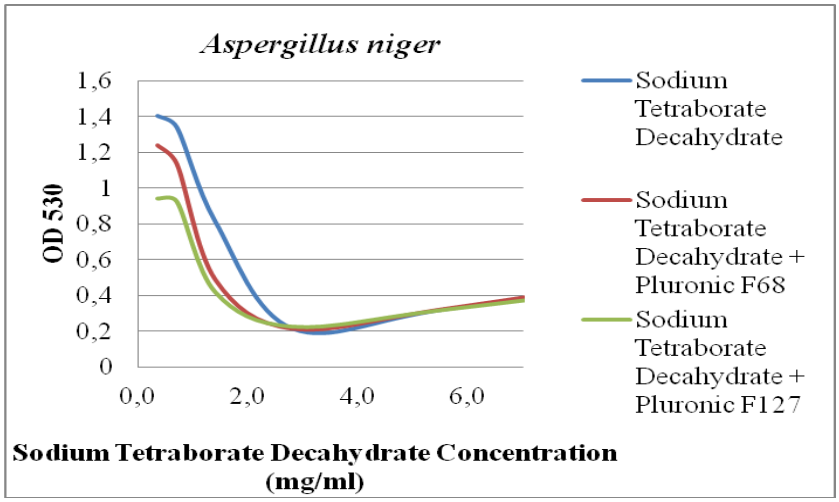

Figure 3.35. Antimicrobial activity of Sodium tetraborate decahydrate and Sodium tetraborate decahydrate -Pluronic F68 and F127 against *A. niger*. Negative control (Sabouraud dextrose broth)

3.3.5. Disodium Octaborate Tetraborate, Disodium Octaborate Tetraborate+ Pluronic F68 and Pluronic F127 Combinations Antimicrobial Susceptibility Test

When DOT antimicrobial effects were investigated on microorganisms, the results showed that DOT showed more antimicrobial activity on yeast and fungi compare to bacteria strains. When bacteria strains treated with DOT, it inhibited growth 86 per cent on *A. baumanii* (Figure 3.36), 36 per cent on *E. coli* (Figure 3.37), 75.06 per cent on *P.aeruginosa* (Figure 3.38), 16 per cent on *S. aureus* (Figure 3.39) and 86 per cent on *B. cereus* (Figure 3.40), respectively. When combined DOT with pF68 and pF127, only DOT+pF68 combination increased 25 per cent inhibition on *S. aureus* (Figure 3.39). Secondly, antimicrobial susceptibility test performed on yeast and fungi cells. According to the data, DOT inhibited growth 76 per cent on *C. albicans* (Figure 3.41), 36 per cent on *C. glabrata* (Figure 3.42), 50 per cent on *K. marxianus* (Figure 3.43) and 21 per cent on *A. niger* (Figure 3.44), respectively. pF68 and pF127 combination with DOT increased percentage of inhibition of growth 24 per cent and 57 per cent for *C. albicans*, 41 per cent and 42 per cent for *C. glabrata*, 14 per cent and 31 per cent and 42 per cent and 30 per cent for *K. marxianus*, respectively (Table 3.6).

Table 3.6. Percentages of growth inhibition for Disodium octaborate tetrahydrate(DOT) and Pluronic F68, F127 treated microorganisms

Microorganism	NC	DOT	F68+DOT	F127+DOT
A. baumanii	0,00	86,63	80,20	72,02
P. aeruginosa	0,00	75,06	73,81	62,19
B. cereus	0,00	7,26	15,59	0,00
S. aureus	0,00	75,06	69,17	50,04
E. coli	0,00	24,72	43,37	31,61
C. albicans	0,00	76,42	96,90	89,76
C. glabrata	0,00	36,88	64,24	87,60
A. niger	0,00	21,68	51,94	64,05
K. marxianus	0,00	50,30	64,60	81,33

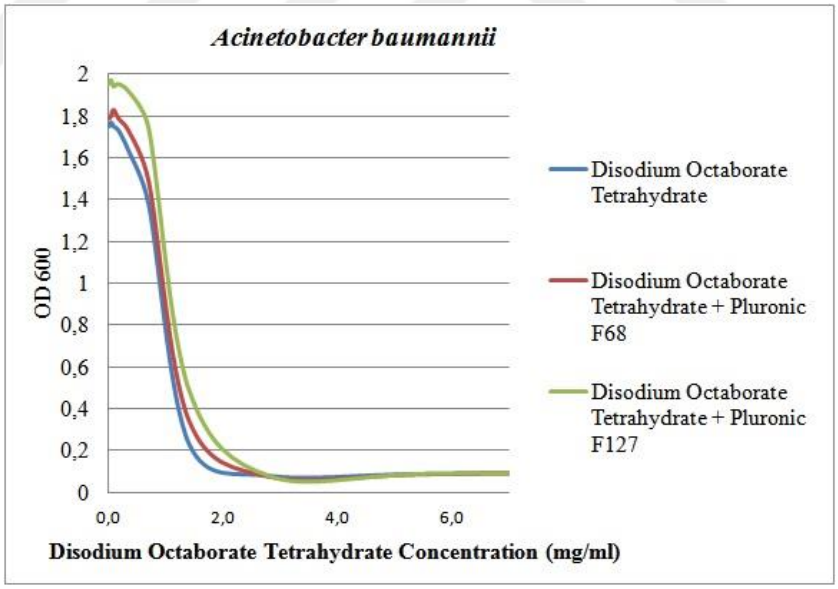

Figure 3.36. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *A. baumannii*. Negative control (Tryptic soy broth)

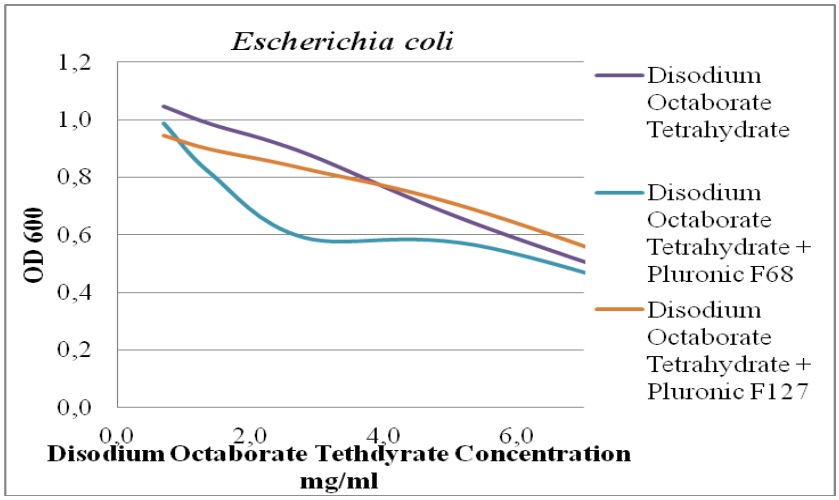

Figure 3.37. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *E.coli*. Negative control (Tryptic soy broth)

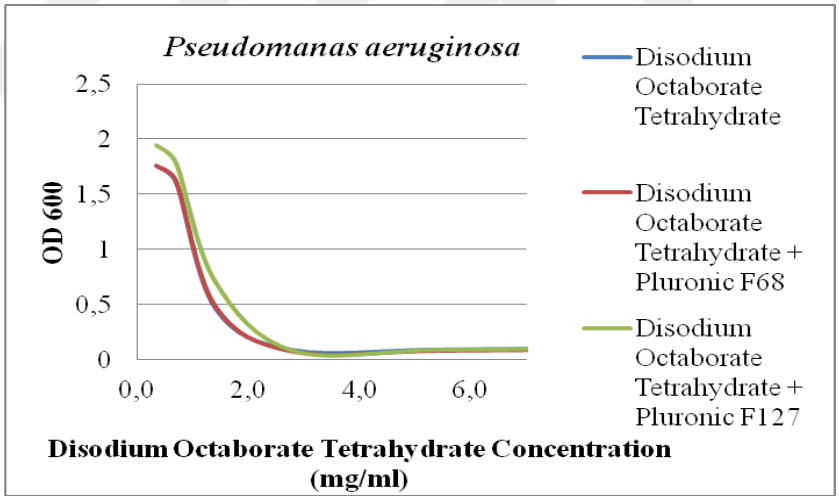

Figure 3.38. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *P. aeruginosa*. Negative control (Tryptic soy broth)

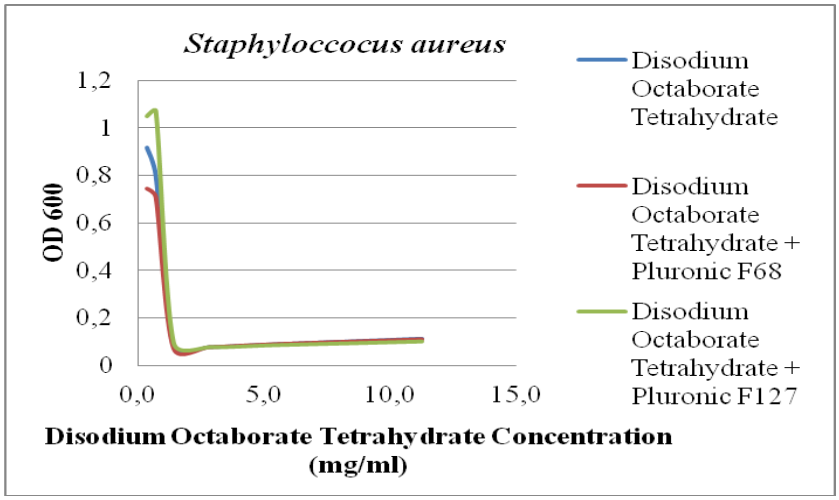

Figure 3.39. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *S. aureus*. Negative control (Tryptic soy broth)

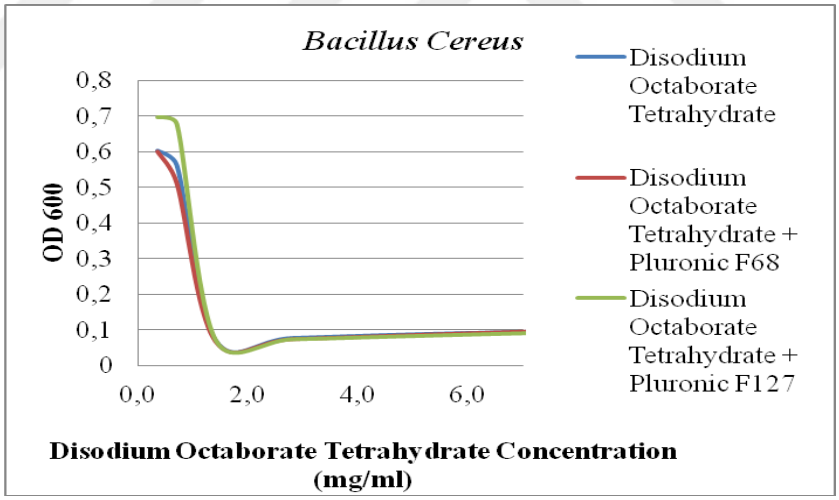

Figure 3.40. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *B. cereus*. Negative control (Tryptic soy broth)

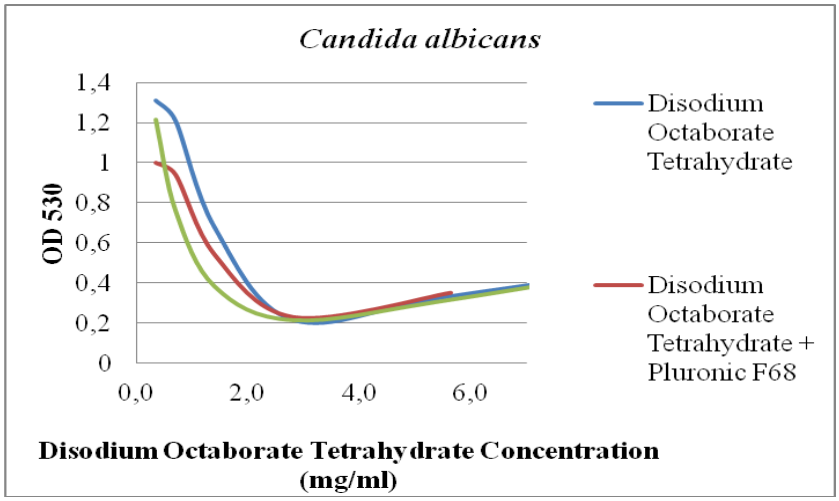

Figure 3.41. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *C. albicans*. Negative control (Sabouraud dextrose broth)

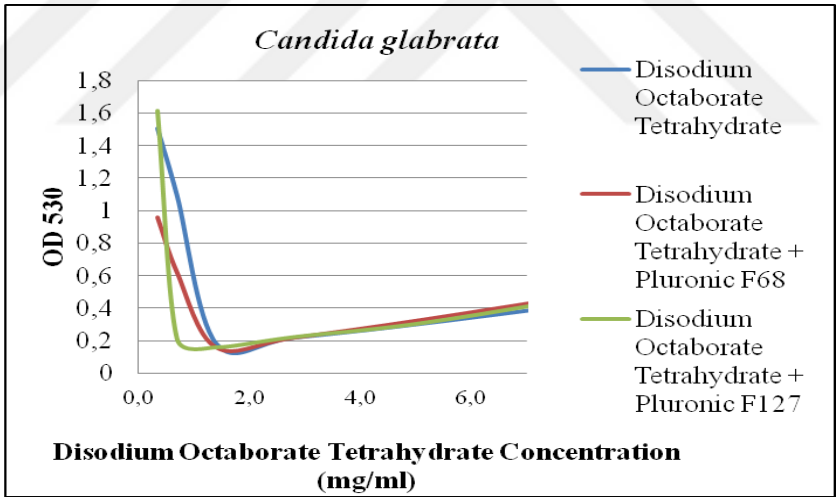

Figure 3.42. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *C. glabrata*. Negative control (Sabouraud dextrose broth)

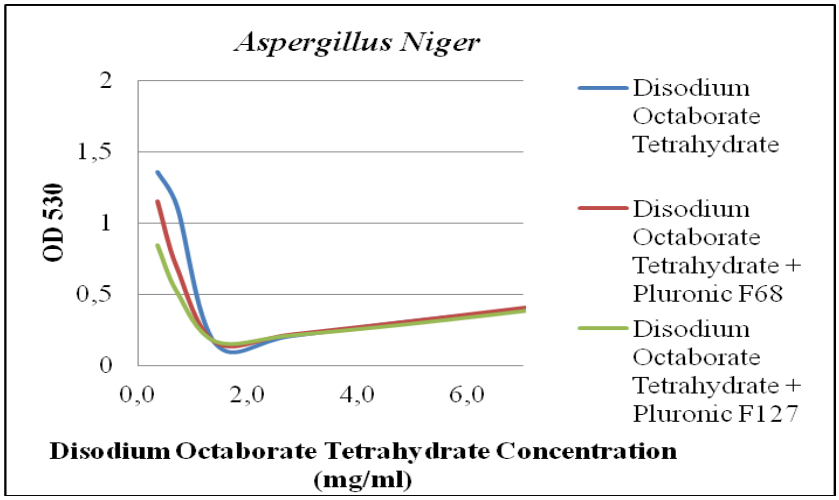

Figure 3.43. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *A. niger*. Negative control (Sabouraud dextrose broth)

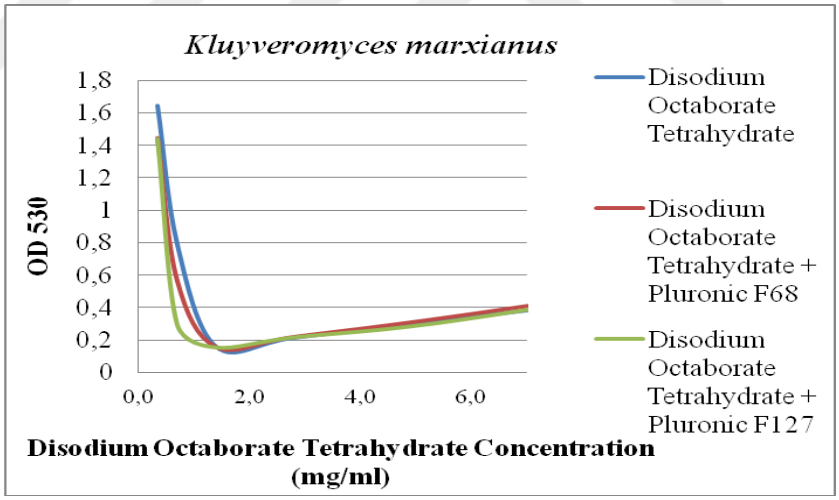

Figure 3.44. Antimicrobial activity of Disodium octaborate tetrahydrate and Disodium octaborate tetrahydrate -Pluronic F68 and F127 against *K. marxianus*. Negative control (Sabouraud dextrose broth)

3.4. ANTIMICROBIAL DIAPER TEST

After determination of antimicrobial effects of boron derivatives, the most effective boron derivative was found as disodium octaborate tetrahdyrate (Table 3.7). Additionally,

DOT+pF68 combination was detected to be highly effective against pathogenic yeast and fungal microorganisms tested (Table 3.8). Therefore, DOT and pF68 combination was chosen as antimicrobial agent for antimicrobial diaper production. Antimicrobial tests on diapers were performed according to procedure described in materials and methods section. According to results, DOT showed 99.99 per cent inhibition against *S. aureus*, 99.96 inhibition against *P.aeruginosa* and 94,93 per cent inhibition against *A. baumanii* (Table 3.8). However, DOT was not able to inhibit growth of *E. coli* and *B. cereus* (Data not shown). In addition, DOT application exhibited 99.99 per cent inhibition against *C. albicans, K. marxianus, A. niger* but effect on *C. glabrata* was found to be 99,98 per cent. DOT combination with pF68 contributed antimicrobial effect especially on bacteria and yeast which were not completely inhibited by DOT alone.

Table 3.7 Percentages of growth inhibition for Disodium octaborate tetrahydrate(DOT) treated diaper which contains microorganisms

Microorganism	**Number of Cells (NC)**	**Number of Cells (640mg)**	**% Inhibition**
A. baumanii	1,20E+11	6,08E+09	94,93
P. aeruginosa	6,80E+10	3,00E+07	99,96
S. aureus	6,82E+10	0,00E+00	99,99
C. albicans	1,20E+10	0,00E+00	99,99
C. glabrata	5,28E+10	8,00E+06	99,98
K.marxianus	2,90E+10	0,00E+00	99,99
A. niger	6,04E+10	0,00E+00	99,99

Table 3.8 Percentages of growth inhibition for Disodium octaborate tetrahydrate(DOT) and Pluronic F68, F127 treated diaper which contains microorganisms

Microorganism	**Number of Cell (NC)**	**Number of Cell (640 mg+320 mg)**	**% Inhibition**
A. baumanii	6,82E+10	0,00E+00	99,99
P. aeruginosa	6,80E+10	0,00E+00	99,99
S. aureus	1,20E+10	0,00E+00	99,99
C. albicans	1,37E+11	0,00E+00	99,99
C. glabrata	2,30E+10	0,00E+00	99,99
K. marxianus	3,36E+10	0,00E+00	99,99
A. niger	6,04E+10	0,00E+00	99,99

4. DISCUSSION

The disposable absorbent diaper market covers baby care, feminine hygiene and adult incontinence managements. Diaper technology has advanced extremely during the last decade and research continues to focus on eliminating skin irritation. Moreover, researchers have developed a model that explains how diaper interact with the skin and cause diaper dermatitis [5]. DD may appear after a cascade of events in which skin is exposed to overhydration, frictional damage and increase of pH of urine and feces mixture. These events weaken the barrier function of the stratum corneum [65], trigger inflammatory and repair mechanism, and subsequently elevated microbial counts pass inside epidermis. Clinical studies have evaluated interventions for the prevention and treatment of DD including skin cleansing (perineal skin cleanser, soap and water) and protectants. Although, this prevention method decreases pH level of skin, it has to be applied to the patient skin for every time diaper used to prevent DD [66]. Moreover, these types of cleansing agents should be applied gently to the skin for prevention of skin irritation. The moisturizing skin products are other agents to prevent DD. They are useful to repair skin barrier and reduce trans-epidermal water loss. However, moisturizer formulations can lead to allergic contact dermatitis and also moist might increase microbial colonization in skin area [67]. Skin protector agents have been used as another alternative treatment method to prevent patients from DD. Many protective agents are based on occlusive substances thus possess as skin protection function, but some types of petroleum-based ointments may show irritation on skin. Moreover, Zinc oxide-based ointments shows poor skin hydration and barrier function to prevent maceration of skin [68]. All of these agents also remain ineffective for inhibition microbial colonization in diaper and skin area. As a result, limited methods for prevention and treatment of diaper dermatitis prevention showed the necessity of new agents for hygienic diapers [69]. The important point for hygienic diaper design is the inhibition microorganism development and colonization in the diaper area. In this research, the main objective of developing new hygienic diaper is inhibition microorganism activity for colonization in diaper area. Another important point for diaper design is development of antifungal, anticandidal and antibacterial formulation to inhibit microbial growth on diaper and skin area. According to researches about antibacterial agents used for killing bacteria strains, may lead to overgrowth of *Candida*

species. For example, a research on amoxicillin antibacterial agent, decreased number of bacterial strains, isolated from diaper area, increased the recovery of *C. albicans* isolates [70]. Thus, development anti-infectious agents to inhibit all types of microorganism are essential point for development of hygienic diaper.

In the current study, boron derivatives were found to be promising antimicrobial agent for inhibition of microorganism growth in the diaper area. Boron derivatives exhibited a wide range of antimicrobial (antibacterial, antifungal and anticandidal) activity against bacterial, fungal and yeast species. Modified disc diffusion assay results showed that the antimicrobial effects of boron derivatives were different for each microorganism. According to the results, antibacterial activity of DOT were found to be greater than BA, STD, ZB and STD against *B. cereus, E. coli*, *S. aureus*, *P. aeruginosa* and *A. baumanii*. Additionally, modified disc diffusion test displayed that direct interaction of powder form of boron derivatives with microorganisms can provide complete inhibition. Hubbard has shown that secure concentration for powdered form of boron derivatives found as between 2000 mg/kg to 4500 mg/kg in rats. Therefore, there is no evidence found to result in skin irritation on human by a powdered form of boron derivatives in this range [49]. Thus, powder form of boron derivatives might be potential antimicrobial agent for diaper production if powder form of boron derivatives is used in safe concentration. Our findings showed that all boron derivatives exhibited antimicrobial activity in safe concentration ranges. Another important point for diaper production is that after diaper is contaminated (urine and feces) by patient usage and starts to interact with patient's skin, safe concentration of antimicrobial agent must be dissolved in the contaminated area and inhibit growth of microorganism to prevent possible DD problem. Therefore, powder form of the boron derivatives were dissolved in water and medium. Aqueous solutions of boron derivatives dissolved in water and in medium have also displayed remarkable antimicrobial effects in safe concentrations. Sayın and co-workers have shown that MIC value for BA and DOT for bacteria (*S. aureus ATCC 25923* and *P. aeruginosa ATCC 27853*) have been different (0.77 to 3.09 mg/ml) as it was found in this study [43]. In the same study, MIC levels of DOT were found to be in different ranges in comparison with this study.

As a different approach of the present study, antifungal properties of all boron derivatives have been found to be higher than their antibacterial activity. Meers and co-workers published that 10 g/l and 20 g/l of BA can be used as an alternative treatment for vaginal

candidiasis [71]. For that reason, BA was tested as a possible antimicrobial agent to be used in the development of antimicrobial adult diaper, but data showed that DOT had significant antimicrobial effect against fungal and yeast species compared to other types of boron derivatives. DOT has highly effective even at low concentrations against *C. albicans* which is the most common cause of secondary dermatitis. Although both boron derivatives have antimicrobial effects against microorganisms tested, some microorganisms showed resistance against boron derivatives. Therefore, pluronics used as antimicrobial drug carrier to decrease MIC level and increase antimicrobial activity of boron derivatives. In this sense, boron derivatives-pF68 and pF127 combinations were used to increase percentage of growth inhibition. The result showed that boron derivatives combined with pF68 and pF127 have a unique antimicrobial effect on each microorganism. Moreover, both boron derivatives combined with pF68 and pF127 displayed greater antimicrobial activity with respect to all boron derivatives. Especially, DOT and pF68 combination showed remarkable inhibition against all bacterial, fungal and yeast species. Dogan and co-workers showed that two per cent *(w/v)* pluronics (pF68 and pF127) combined with sodium pentaborate pentahydrate were found to be effective in wound healing treatment along with providing inhibition of microbial growth in the wound area [72]. In line with these results, findings of the current study showed that antimicrobial effects of boron combinations with pluronics were found to be similar to that study. As a conclusion, DOT + pF68 combination was chosen as possible promising antimicrobial formulation because this combination is safe, non-toxic, cheap, displaying broad-range antimicrobial properties, easily dissolve in aqueous solutions and is applicable to diaper production process. To relevance MIC data for this combination to use on adult diaper is said to be hard to interpolate, so additional study was done in test antimicrobial performance of the diaper. Although using classical laboratory microorganisms helpful to get valuable data, it does not reflect the real world condition of diaper. However, using these methods, described in the present study, can stimulate real world conditions. After method applied to pathogens, results showed that differences between treated and untreated percentage of inhibition of growth have been found to be 99 per cent except *B. cereus*, *E. coli* and *C. glabrata* species. It was previously proven that some yeast species can tolerate high level of boron by a regulation of ATR1, boron transporter gene, gene expression [73]. Therefore, one possible explanation for remaining ineffective against *C. glabrata* would be high expression levels of ATR1. Moreover, bacterial species have also ATR1-like drug

transporter mechanisms, ATP binding cassette (ABC) transporter, and *E. coli* and *B. cereus* were found to be the most resistant bacteria against all types of boron derivatives [74]. Although, ABC transporter mechanism considering as the responsible mechanism for boron tolerance, this hypothesis waits to be clarified to understand the roles of transporters in boron resistance.

To sum up, DOT and pF68 combination is a promising antimicrobial formulation for the production of hygienic diaper, however it is not effective against boron resistant yeast and bacterial species. Boron derivative combinations against resistant microorganism can be a useful strategy to solve boron resistance problem or some antimicrobial agents can be combined with boron to treat resistant yeast infection in the diaper area. In addition, anti-allergic properties of boron might be investigated in a dose dependent manner to provide anti-allergic and antimicrobial properties at the same time to prevent allergic diaper dermatitis. Moreover, skin sensitization and irritation must be investigated in detail before producing hygienic APD for consumers.

5. CONCLUSION

In today's technology, technological advances in development of diaper design have presented more compelling products which decrease skin overhydration and friction damage to the skin. The most important development for disposable diaper was production of diaper containing absorbent gelling material used in diaper to decrease DD rate. However, microorganism-related dermatitis problems are still remaining to be solved in the diaper area. In this manner, antimicrobial agents are urgently required in diaper design to prevent colonization of microorganism in the diaper area. Therefore, boron was used as antimicrobial agent to inhibit microbial colonization in the diaper area. Overall data suggest that boron derivatives, especially DOT, are potential antimicrobial agents to be used in hygienic diaper production to decrease the microorganisms-related DD. However, there are still much works on skin sensitization and irritation to realize these chemicals in markets.

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APPENDIX A: MIS RESULTS FOR MICROORGANISMS

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.6922	1.194E+6	0.005	----	6.6807		----	< min rt	
0.7000	9.646E+8	0.017	----	6.7394	SOLVENT PEAK	----	< min rt	
0.7669	9101	0.016	----	7.2430		----	< min rt	
0.7927	849	0.011	----	7.4370		----	< min rt	
1.1000	513	0.010	----	9.7489		----		
1.4215	490	0.009	----	11.5446		----		
1.5184	311	0.009	1.048	12.0013	12:0	0.42	ECL deviates 0.001	Reference -0.004
1.6726	4002	0.008	1.020	12.6211	13:0 iso	5.22	ECL deviates -0.002	Reference -0.009
1.6956	514	0.009	1.016	12.7135	13:0 anteiso	0.67	ECL deviates -0.001	Reference -0.008
1.8495	429	0.009	----	13.2970		----		
1.9417	2249	0.008	0.984	13.6278	14:0 iso	2.83	ECL deviates 0.000	Reference -0.010
2.0460	2567	0.009	0.973	14.0018	14:0	3.20	ECL deviates 0.002	Reference -0.009
2.2335	26810	0.008	0.958	14.6317	15:0 iso	32.87	ECL deviates 0.000	Reference -0.012
2.2614	2378	0.009	0.956	14.7251	15:0 anteiso	2.91	ECL deviates 0.000	Reference -0.011
2.3198	539	0.010	0.952	14.9209	15:1 w5c	0.66	ECL deviates -0.005	
2.3446	634	0.009	0.951	15.0042	15:0	----	ECL deviates 0.004	
2.5009	2115	0.009	0.942	15.5076	Sum In Feature 2	2.55	ECL deviates -0.008	14:0 3OH/16:1 iso I
2.5399	6490	0.010	0.941	15.6329	16:0 iso	7.81	ECL deviates 0.000	Reference -0.012
2.6153	4982	0.009	0.938	15.8759	Sum In Feature 3	5.98	ECL deviates 0.001	16:1 w6c/16:1 w7c
2.6544	8012	0.009	0.936	16.0016	16:0	9.60	ECL deviates 0.002	Reference -0.011
2.7831	1245	0.010	0.932	16.4128	17:1 iso w10c	1.48	ECL deviates -0.001	
2.8051	4270	0.010	0.931	16.4833	17:1 iso w5c	5.09	ECL deviates 0.000	
2.8321	783	0.010	0.930	16.5694	17:1 anteiso A	0.93	ECL deviates -0.002	
2.8526	8853	0.009	0.930	16.6351	17:0 iso	10.53	ECL deviates -0.002	Reference -0.015
2.8828	1212	0.010	0.929	16.7316	17:0 anteiso	1.44	ECL deviates -0.001	Reference -0.014
2.9672	356	0.010	0.927	17.0013	17:0	0.42	ECL deviates 0.001	Reference -0.011
3.2141	589	0.012	0.923	17.7927	18:1 w9c	0.70	ECL deviates -0.001	
3.2789	3970	0.010	0.922	18.0004	18:0	4.68	ECL deviates 0.000	Reference -0.012
3.7142	439	0.012	----	19.4277		----		
4.1318	422	0.011	----	20.8240		----	> max rt	
----	2115	---	----	----	Summed Feature 2	2.55	12:0 aldehyde ?	unknown 10.9525
----	-----	---	----	----		----	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I
----	4982	---	----	----	Summed Feature 3	5.98	16:1 w7c/16:1 w6c	16:1 w6c/16:1 w7c

ECL Deviation: 0.002
Total Response: 84117
Percent Named: 97.78%

Reference ECL Shift: 0.011
Total Named: 82246
Total Amount: 78743

Number Reference Peaks: 13

Matches:

Library	Sim Index	Entry Name
RCLIN6 6.00	0.455	Bacillus-cereus-GC subgroup C
	0.400	Bacillus-thuringiensis-GC subgroup B
	0.387	Bacillus-cereus-GC subgroup D
	0.289	Bacillus-mycoides

Figure A.1. GC identification system was used to identify *B. cereus*

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.6925	124275	0.005	----	6.6855		----	< min rt	
0.7006	1.022E+9	0.017	----	6.7464	SOLVENT PEAK	----	< min rt	
0.7913	843	0.010	----	7.4282		----	< min rt	
1.5180	5325	0.009	1.057	12.0001	12:0	9.35	ECL deviates 0.000	Reference -0.008
1.6429	474	0.011	1.032	12.5019	unknown 12.502	----	ECL deviates 0.000	
1.8243	2437	0.009	1.002	13.2049	12:0 2OH	4.05	ECL deviates 0.001	
1.9025	3589	0.009	0.992	13.4848	12:0 3OH	5.91	ECL deviates 0.002	
2.0464	411	0.009	0.974	13.9999	14:0	0.66	ECL deviates 0.000	Reference -0.007
2.1967	532	0.010	0.960	14.5039	Sum In Feature 1	0.85	ECL deviates 0.001	13:0 3OH/15:1 iso H
2.3451	627	0.009	0.949	15.0010	15:0	----	ECL deviates 0.001	
2.5057	2997	0.009	0.939	15.5186	Sum In Feature 2	4.67	ECL deviates 0.003	14:0 3OH/16:1 iso I
2.5916	584	0.007	0.934	15.7955	16:1 w9c	0.91	ECL deviates -0.005	
2.6044	5180	0.009	0.933	15.8369	Sum In Feature 3	8.03	ECL deviates -0.003	16:1 w7c/16:1 w6c
2.6549	12890	0.009	0.931	15.9996	16:0	19.92	ECL deviates 0.000	Reference -0.007
2.9095	2648	0.009	0.921	16.8122	17:1 w8c	4.05	ECL deviates -0.003	
2.9684	1218	0.009	0.920	17.0003	17:0	1.86	ECL deviates 0.000	Reference -0.006
3.2153	26203	0.010	0.914	17.7904	18:1 w9c	39.75	ECL deviates -0.004	
----	532	---	----	----	Summed Feature 1	0.85	15:1 iso H/13:0 3OH	13:0 3OH/15:1 iso H
----	2997	---	----	----	Summed Feature 2	4.67	12:0 aldehyde ?	unknown 10.9525
----	-----	---	----	----		----	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I
----	5180	---	----	----	Summed Feature 3	8.03	16:1 w7c/16:1 w6c	16:1 w6c/16:1 w7c

ECL Deviation: 0.002 Reference ECL Shift: 0.007 Number Reference Peaks: 4
Total Response: 64012 Total Named: 64012
Percent Named: 100.00% Total Amount: 61325

Matches:

Library	Sim Index	Entry Name
RCLIN6 6.00	0.485	Acinetobacter-baumannii
	0.366	Acinetobacter-haemolyticus

Figure A.2. GC identification system was used to identify *A. baumanii*

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.6925	372581	0.005	----	6.6820		----	< min rt	
0.7006	1.022E+19	0.017	----	6.7432	SOLVENT PEAK	----	< min rt	
1.1314	951	0.011	1.194	9.9832	10:0	0.60	ECL deviates -0.017	
1.2164	749	0.010	----	10.4822		----		
1.3475	491	0.009	----	11.2014		----		
1.4013	5063	0.010	1.089	11.4534	10:0 3OH	2.90	ECL deviates 0.005	
1.5181	11427	0.009	1.057	12.0005	12:0	6.36	ECL deviates 0.000	Reference -0.007
1.6357	469	0.011	1.034	12.4721	11:0 3OH	0.25	ECL deviates 0.007	
1.8245	18372	0.008	1.002	13.2041	12:0 2OH	9.68	ECL deviates 0.000	
1.9027	15598	0.008	0.992	13.4841	12:0 3OH	8.14	ECL deviates 0.001	
2.0465	865	0.009	0.974	13.9987	14:0	0.44	ECL deviates -0.001	Reference -0.007
2.3451	1477	0.009	0.949	14.9995	15:0	----	ECL deviates 0.000	
2.6043	14458	0.009	0.933	15.8359	Sum In Feature 3	7.10	ECL deviates -0.004	16:1 w7c/16:1 w6c
2.6553	61425	0.009	0.931	16.0005	16:0	30.09	ECL deviates 0.001	Reference -0.006
2.9096	543	0.009	0.921	16.8134	17:1 w8c	0.26	ECL deviates -0.002	
2.9406	5040	0.009	0.920	16.9126	17:0 cyclo	2.44	ECL deviates -0.002	
2.9683	772	0.008	0.920	17.0012	17:0	0.37	ECL deviates 0.001	Reference -0.007
3.2312	50179	0.009	0.914	17.8445	Sum In Feature 8	24.11	ECL deviates -0.003	18:1 w7c
3.2794	1522	0.009	0.913	17.9991	18:0	0.73	ECL deviates -0.001	Reference -0.011
3.5635	13639	0.009	0.909	18.9319	19:0 cyclo w8c	6.52	ECL deviates 0.000	
----	14458	---	----	----	Summed Feature 3	7.10	16:1 w7c/16:1 w6c	16:1 w6c/16:1 w7c
----	50179	---	----	----	Summed Feature 8	24.11	18:1 w7c	18:1 w6c

ECL Deviation: 0.005
Reference ECL Shift: 0.008
Number Reference Peaks: 5
Total Response: 201564
Total Named: 200324
Percent Named: 99.38%
Total Amount: 191489

Matches:

Library	Sim Index	Entry Name
RCLIN6 6.00	0.584	Pseudomonas-aeruginosa
	0.465	Pseudomonas-aeruginosa-mucoid strains

Figure A.3. GC identification system was used to identify *P. aeruginosa*

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.6923	660465	0.005	----	6.6811		----	< min rt	
0.7004	1.01E+9	0.017	----	6.7416	SOLVENT PEAK	----	< min rt	
0.7914	898	0.010	----	7.4260		----	< min rt	
1.2963	416	0.010	1.121	10.9477	Sum In Feature 2	0.36	ECL deviates -0.005	unknown 10.9525
1.5183	5560	0.009	1.057	12.0003	12:0	4.57	ECL deviates 0.000	Reference -0.006
1.7677	1236	0.009	1.010	12.9997	13:0	0.97	ECL deviates 0.000	Reference -0.006
2.0001	311	0.009	----	13.8322		----		
2.0469	9721	0.009	0.974	13.9998	14:0	7.36	ECL deviates 0.000	Reference -0.005
2.1981	1904	0.011	0.960	14.5065	Sum In Feature 1	1.42	ECL deviates 0.003	13:0 3OH/15:1 iso H
2.3455	6259	0.009	0.949	15.0002	15:0	----	ECL deviates 0.000	
2.4007	349	0.008	----	15.1784		----		
2.5059	12428	0.009	0.939	15.5177	Sum In Feature 2	9.06	ECL deviates 0.002	14:0 3OH/16:1 iso I
2.6047	20667	0.009	0.933	15.8365	Sum In Feature 3	14.99	ECL deviates -0.004	16:1 w7c/16:1 w6c
2.6553	35615	0.009	0.931	15.9997	16:0	25.76	ECL deviates 0.000	Reference -0.006
2.8215	436	0.011	0.924	16.5306	15:0 3OH	0.31	ECL deviates -0.002	
2.9100	862	0.010	0.921	16.8136	17:1 w8c	0.62	ECL deviates -0.001	
2.9406	14084	0.009	0.920	16.9113	17:0 cyclo	10.07	ECL deviates -0.004	
2.9687	4664	0.009	0.920	17.0012	17:0	3.33	ECL deviates 0.001	Reference -0.005
3.2313	26697	0.009	0.914	17.8427	Sum In Feature 8	18.95	ECL deviates -0.005	18:1 w7c
3.2494	432	0.009	0.913	17.9004	Sum In Feature 8	0.31	ECL deviates -0.002	18:1 w6c
3.2802	541	0.010	0.913	17.9994	18:0	0.38	ECL deviates -0.001	Reference -0.009
3.5639	2182	0.010	0.909	18.9298	19:0 cyclo w8c	1.54	ECL deviates -0.002	
----	1904	---	----	----	Summed Feature 1	1.42	15:1 iso H/13:0 3OH	13:0 3OH/15:1 iso H
----	12843	---	----	----	Summed Feature 2	9.43	12:0 aldehyde ?	unknown 10.9525
----	-----	---	----	----		----	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I
----	20667	---	----	----	Summed Feature 3	14.99	16:1 w7c/16:1 w6c	16:1 w6c/16:1 w7c
----	27130	---	----	----	Summed Feature 8	19.25	18:1 w7c	18:1 w6c

ECL Deviation: 0.003 Reference ECL Shift: 0.006 Number Reference Peaks: 6
Total Response: 138104 Total Named: 137445
Percent Named: 99.52% Total Amount: 134658

Matches:

Library	Sim Index	Entry Name
RCLIN6 6.00	0.901	Kluyvera-cryocrescens
	0.804	Shigella-boydii-GC subgroup B (high DNA homol. with E. coli)
	0.664	Salmonella-enteritidis
	0.625	Klebsiella-pneumoniae-pneumoniae-GC subgroup B
	0.606	Escherichia-coli-GC subgroup A (high DNA homol. with Shigella)
	0.590	Enterobacter-cloacae-GC subgroup A
	0.547	Salmonella-choleraesuis-choleraesuis

Figure A.4. GC identification system was used to identify *E. coli*

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.6921	787598	0.004	----	6.6831		----	< min rt	
0.6997	9.78E+8	0.017	----	6.7404	SOLVENT PEAK	----	< min rt	
0.7614	1446	0.013	----	7.2044		----	< min rt	
0.7923	1036	0.012	----	7.4367		----	< min rt	
1.0996	888	0.010	----	9.7468		----		
1.4214	869	0.009	----	11.5480		----		
1.8492	649	0.009	----	13.2964		----		
1.9418	502	0.008	0.983	13.6279	14:0 iso	0.30	ECL deviates 0.000	Reference -0.012
2.2341	11316	0.008	0.962	14.6315	15:0 iso	6.71	ECL deviates 0.000	Reference -0.012
2.2621	76451	0.008	0.961	14.7255	15:0 anteiso	45.28	ECL deviates 0.001	Reference -0.011
2.3193	603	0.009	0.958	14.9172	15:1 w5c	0.36	ECL deviates -0.009	
2.5367	3302	0.012	0.949	15.6213	16:0 iso	1.93	ECL deviates -0.012	
2.6544	2759	0.009	0.946	16.0004	16:0	1.61	ECL deviates 0.000	Reference -0.012
2.7997	581	0.010	----	16.4657		----		
2.8531	9881	0.009	0.941	16.6367	17:0 iso	5.73	ECL deviates 0.000	Reference -0.013
2.8831	41730	0.009	0.941	16.7328	17:0 anteiso	24.20	ECL deviates 0.000	Reference -0.013
3.1653	702	0.009	0.937	17.6358	18:0 iso	0.41	ECL deviates 0.000	Reference -0.013
3.2793	8471	0.010	0.936	18.0003	18:0	4.89	ECL deviates 0.000	Reference -0.013
3.4740	3165	0.009	0.935	18.6365	19:0 iso	1.82	ECL deviates -0.001	
3.5050	7889	0.010	0.935	18.7379	19:0 anteiso	4.55	ECL deviates 0.000	
3.7143	569	0.010	----	19.4309		----		
3.8849	3869	0.011	0.932	20.0000	20:0	2.22	ECL deviates 0.000	Reference -0.014
4.1309	625	0.011	----	20.8210		----	> max rt	

ECL Deviation: 0.004
Total Response: 174196
Percent Named: 97.96%

Reference ECL Shift: 0.013
Total Named: 170640
Total Amount: 162211

Number Reference Peaks: 9

Matches:

Library	Sim Index	Entry Name
RCLIN6 6.00	0.694	Staphylococcus-haemolyticus
	0.593	Staphylococcus-aureus-GC subgroup G (USP strain)
	0.563	Staphylococcus-capitis-capitis
	0.558	Staphylococcus-lugdunensis
	0.523	Staphylococcus-warneri-GC subgroup A
	0.446	Staphylococcus-aureus-GC subgroup C

Figure A.5. GC identification system was used to identify *S. aureus*