Available online at http://ijcpe.uobaghdad.edu.iq and www.iasj.net
Iraqi Journal of Chemical and Petroleum Engineering
Vol. 24 No.1 (March 2023) 105 – 112
EISSN: 2618-0707, PISSN: 1997-4884

Testing of a Potentially Used Antiseptic Consists of Povidon Iodine, Hydrogen Peroxide and Aloe Vera

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Abstract

In this study a new antiseptic was formulated and tested to match the effectiveness against microorganisms. The formulation consisted of Povidone - Iodine (PVP-I) (10%), H_2O_2 (3%) and Aloe Vera gel (pure). Different ratios of these materials were prepared within the acceptable range of pH for an antiseptic (3-6). The prepared samples were tested. The In Vitro test was performed by using four bacteria, two were Gram-Positive (Staphylococcus aureus and Bacillus cereus) and two were Gram-Negative (Escherichia coli and Pseudomonas aeruginosa). The new antiseptic showed 100% killing rate for E. coli, Ps. aeruginosa and S. aureus and 96.4667% killing rate for B. cereus. When the new antiseptic was compared with two common antiseptics (chloroxylenol (Dettol) and alcohol-based antiseptic), it was superior because the alcoholic antiseptic showed 100% killing rate for E. coli, Ps. aeruginosa and S. aureus and 89.8000% for B. cereus. The Dettol did not show killing rate against bacteria. Ex vivo test was carried out using the sample that showed the highest effectiveness in the In Vitro test. This was performed by applying the formulation on the skin of lab mice after wounding and contaminating the wounds with two bacteria (Staphylococcus aureus and Escherichia coli). After applying the antiseptic on the wounds, swabs from the wounds were taken for testing. The new antiseptic showed amazing efficacy against bacteria by leaving agar dish completely empty from bacteria. In vivo test was also conducted using the polymerase chain reaction (PCR) test for COVID-19. The new antiseptic did not show effectiveness against Coronavirus because the virus could not be isolated like bacteria.

Keywords: Aloe Vera, Antiseptic, Ex Vivo, Hand hygiene, In Vitro, In Vivo, Povidone - Iodine.

Received on 30/08/2022, Received in Revised Form on 24/10/2022, Accepted on 26/10/2022, Published on 30/03/2023

https://doi.org/10.31699/IJCPE.2023.1.12

1- Introduction

An antiseptic is a substance that stops or slows down the growth of microorganisms. Hand hygiene is recommended by WHO guidelines, which includes frequent hand washing with soap and water for at least 20 seconds after using the restroom, before eating, and after coughing, sneezing, or blowing one's nose. The Food and Drug Administration (FDA) recommends sanitizing non-visible soiled hands with an alcohol-based agent containing 80 percent v/v ethanol or 75 percent v/v isopropanol when soap and water are unavailable [1]. Also, these guidelines published the activities of common used antiseptics and disinfectants with their mechanisms of action on different microorganisms, i.e. bacteria, viruses, fungi and spores. Many of these known antiseptics have limitations in inactivating one or more of these kinds of microorganisms. Others were found to be ineffective or harmful to humans like triclosan [2]. Hand sanitization's success is entirely reliant on the use of effective hand disinfecting agents, which come in a variety of types and forms, including antimicrobial soaps, water-based and alcohol-based hand sanitizers [3]. Alcohol is a common antiseptic due to its low cost and ease of production, but it appears to be less beneficial than PVP-I at killing bacteria [4].

Povidone-iodine (PVP-I) is a well - known alternative antiseptic to alcohol that is commonly used for skin antisepsis before and after surgery as well as in clinical settings. It is sometimes applied to the skin as a liquid or a powder and it may be used to treat instantaneous infections as well as preventing the spread of opportunistic diseases. Povidone-iodine or Betadine is Polyvinylpyrrolidone-iodine (PVP-I) a chemical that is widely used as an antiseptic [5]. It consists of molecular iodine and polyvinylpyrrolidone that give it the yellowish-brown to reddish-brown colour. It is amorphous powder which has a characteristic odor. Povidone-iodine has good solubility in water and in alcohol. It is applied on the skin and has a broad microbicidal spectrum against bacteria, viruses, fungi, yeasts, and protozoa [4]. There are ten possible aqueous iodine formulas only three of them have antimicrobial activity with molecular iodine be the most effective one. The formula that iodine takes in its aqueous solutions is greatly influenced by the pH of the medium. The presence of the acidic medium helps shift the reaction to form molecular iodine (I_2) to become the dominant formula at low pH values [6]. According to the Pharmacopoeia, pH of povidone-iodine must be between (1.5 - 5), but the optimal range is between (3-6). This range of pH is

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essential for obtaining the best efficacy of povidone-iodine, and also it ensures the stability of iodine in its complex, i.e. povidone-iodine [7]. The use of sterile aqueous iodine solutions may be accompanied with several problems such as weak solubility in water, chemical instability and potential local injury such as skin irritation. For overcoming all iodine problems, iodine carriers or what is known by iodophors emerged. They are polymers or large organic polymers complexed with iodine, Cadexomer-Iodine and Povidone-Iodine are the most commonly used of iodophors [8]. Although the exact mechanism of the antisepsis effects of iodine is not fully understood, its capability in rapid penetration to the organism's cell wall and binding with proteins is a key factor in these effects. PVP-I has a wide antimicrobial spectrum. It is active against Gram-positive and Gram-negative bacteria as well as antibiotic-resistant and antiseptic-resistant strains so as fungi and protozoa. Its activity extends to a broad range of enveloped and non-enveloped viruses moreover some bacterial spores if the time of exposure is increased. Research has also shown iodine effectiveness in removing Mycobacterium tuberculosis Methicillin-resistant Staphylococcus aureus (MRSA) [9, 10]. As a general rule, the effectivity of antiseptics increases, but the contact time required to reach maximum effectivity decreases as the applied concentrations increases provided other variables are held constants. Studies conducted on PVP-I showed the bias of iodine complexes from this norm. In vitro studies monitored an increase in the amount of free molecular iodine in diluting the povidone-iodine solution (10%) [6]. Basically, PVP-I is well tolerated by the majority of users, especially when applied to the skin [9]. In comparison to other antiseptics, PVP-I does not cause allergy or skin inflammation but anaphylactic and urticarial cases may occur very rarely. Nevertheless, PVP-I may cause thyroid dysfunction with long-term use as mouthwash or gargle. Although its safety for use does not lose its position, long-term patients using PVP-I should be monitored frequently [11].

Hydrogen peroxide (H_2O_2) is commonly used for antisepsis, disinfection, and sterilization. It is a liquid of clear and colorless nature which is found commercially in different concentrations (3% - 90%). Hydrogen peroxide is regarded as a friendly ingredient for environment due to its rapid degradation to the safe terminals; water and oxygen. Hydrogen peroxide proved broad-spectrum efficacy against bacteria, viruses, bacterial spores, and yeasts. In particular, hydrogen peroxide shows greater activity against gram-positive than gram-negative bacteria [8, 12]. The use of hydrogen peroxide-based disinfectants could reduce the concentrations of traditional disinfectants and therefore a reduction of their chemical residues in the environment after being used [12]. The synergistic effect of PVP-I and hydrogen peroxide was proved to have enhanced antimicrobial activity and may overcome acquired microbial resistance to single antiseptic [13]. It was shown that the addition of hydrogen peroxide to povidone-iodine significantly enhanced the effectiveness against plaque and gingivitis than using each product alone in an in vitro model [14]. Also, recently, the combination of PVP-I and H_2O_2 proved its success in irrigation intraoperative wounds to inhibit the infection after spine surgery [15].

Aloe vera is a medicinal plant traditionally used since 1500 BC in many countries such as Greece, China, and Mexico. It also has been used for centuries as a traditional medicine for various diseases and skin lesions. Aloe vera is an indigenous plant from tropical Madagascar, Saudi Arabia, and Iran. It belongs to the Liliaceae family; it is similar to Cactus and is an herbaceous and perennial plant with thick, fleshy and long leaves [16]. Aloe Vera, has been used therapeutically for many centuries and is of particular interest due to its lengthy historic reputation as a curative agent and its widespread use in complementary therapies [16]. The inner, preserved gel is typically used as a health and nutrition supplement or in cosmetics and toiletries after the outer, green cuticle is removed. Nevertheless, very few studies on this ingredient have been done; the majority have used whole leaf extracts. Despite the fact that over 75 active ingredients, including vitamins, enzymes, minerals, lignin, saponins, sugars, sterols, amino acids, salicylic acid, and anthraquinones, have been identified from the inner gel, it has been challenging to link therapeutic benefits to specific active ingredients [17]. It contains over 70 biologically active compounds and is claimed to have anti-inflammatory, anti-oxidant, immune boosting, anticancer, healing, anti-ageing and anti-diabetic properties. Aloes, by contrast, is an anthraquinone derivative of the sap of the Aloe leaf which has been used for centuries as a purgative [18]. The antibacterial activities of A. Vera were dependent on the dose of anthraquinone. It is reported that A. Vera possesses antifungal, antiviral, antibacterial and acaricidal activity against skin infections such as acne, herpes and scabies [19].

European Committee for Standardization (CEN) and Food and Drug Administration (FDA) are the most known protocols for testing hand hygiene [20]. The tests include the following steps:

- In vitro tests include testing of suspension, drawing time - kill curves as well as determining the minimum inhibitory concentrations (MIC) to assess the activity against microbes of hand antiseptics which provide just an exploratory reference of the agents' effect spectrum and speed up of activity.
- Ex vivo tests include testing the formulation on animal's skin or human's. Applying the human skin temperature and times of contact may reflect real cases and represent clearer reference that hand sanitizer is able to treat microorganisms that transmit by hand. It requires testing of hands in the real field for levels of skin contamination with microbes before and after applying the formulation.
- In vivo test is conducted directly on human hands following a certified protocol that simulates very nearly the conditions of using antiseptic in the field which may be followed up by clinical trials. Using these protocols helps to obtain important knowledge

about the activity of a hand sanitizer to cross the diffusion of microbes carried by hands in healthcare environment [20].

These tests based on two main procedures. The first is prepared to assess the hand-rub or the hand-wash formulations' ability to remove transitory microorganisms from the hands of HCWs. This method uses the post-contamination treatment of hands by placing the organism(s) of the test on volunteers' hands then exposing them to the antiseptic. This procedure is important in evaluating the formulations applied in frequent hand sanitization. The second method is conducted especially to the cases of pre-surgery and the purpose is to assess the tested antiseptic's capability to eliminate the inhabitant microbes on the hands [20].

In the present outbreak of COVID-19, searching for an effective hand antiseptic becomes a persistent need all over the world. Since the declaration of COVID-19 as a pandemic on late 2020, The World Health Organization (WHO) and national disease control agencies have repeatedly emphasized the importance of hand hygiene in preventing the spread of the virus. In this respect a study was done to formulate a new antiseptic that met the basic demand of effectiveness against a broader spectrum of microorganisms. The novelty of this work is the formulation of PVP-I, H_2O_2 and Aloe Vera for the first time to prepare hand sanitizer.

2- Experimental Work

2.1. Materials and Chemicals

All Materials and chemicals that have been used are listed in Table 1.

Table 1. Materials and Chemicals Used in the Research

Material Name	Chemical Formula	Purity	Manufacturer Company	Country of Manufacture
Povidone Iodine	$C_6H_9I_2NO$	10%	AQUA Company	Turkey
Hydrogen Peroxide	H_2O_2	3%	Chemical Lab Company	Belgium
Aloe Vera	-	Pure	plant nursery	Iraq

2.2. Equipment

All equipment and devices that were used in the research and their specifications are listed in Table 2. In addition, laboratory glassware (beaker, cylinder, funnel) were also used.

Table 2. Specifications of Equipment and Devices Used in the Research

Equipment Name	Specifications	Manufacturer Company	Country of Manufacture
Magnetic stirrer	0 - 1400 rpm	Heidolph	Germany
pH meter	0 – 14	HM Digital	USA
Blender	0-23000 rpm	Pioneer	Japan

2.3. Procedure

2.3.1. Aloe Vera preparation

Based on what was mentioned in the method of preparing Aloe Vera [21]. the following method was applied. Using cold water to wash the Aloe Vera leaves. Each leaf should have the top and bottom cut off with a large, sharp knife, then discarded. Cutting the leaf in half lengthwise to create two equal-sized pieces. After that, removing the green skin by cutting along the Aloe Vera gel and the skin with a sharp knife. Cutting the Aloe Vera gel and discarding the skin. Then it was mixed with a blender at 23000 rpm for 10 min and then filtered**.**

2.3.2. Formulation preparation

Nine samples were prepared by mixing PVP-I, hydrogen peroxide and Aloe Vera in different ratios. Table 3 shows the specifications of samples. The acidity function, pH, was monitored in the prepared samples to be within the suitable range for living tissue (3-6) [6]. Nine samples were prepared within the acceptable range of pH.

2.3.3. Testing

A- In Vitro Testing

The samples were tested in Food Research Center Lab./ Department of Environmental and Water Treatment / Iraqi Ministry of Science and Technology. Four Kinds of bacteria were selected based on their prevalence in hospitals and health centers. Two were Gram-Positive; Staphylococcus Aureus and Bacillus Cereus and two were Gram-Negative; Escherichia coli and Pseudomonas aeruginosa. The laboratory results showed the number of remaining bacteria after treating with the Samples. The killing rate were calculated according to the following equation:

$$\% \text{ Kill rate} = ((\text{Control} - \text{Residual}))/\text{Control}*\%100 \quad (1)$$

B- Ex Vivo Testing

The best samples which showed maximum killing rate in the In Vitro test (Samples 6 and 7) were directed forward to Ex Vivo testing. After getting the necessary approvals, the test was done at the Biotechnology Research Center / Al-Nahrain University where the experiments were carried out in a laboratory setting right away. This test was applied on animal skin, specifically on the skin of laboratory mice, according to the following procedure. Two types of bacteria were selected and cultivate, Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa). Four mice

were isolated and ensured their safety. An area of mouse skin was sterilized and cleaned with a razor. Small cuts on the skin were made with a razor, then the wound was contaminated with the prepared bacteria, two mice were contaminated with one type of bacteria. They were left for 24 hours. After that the wound was sterilized with the Samples. Two mice were sterilized with the Sample 6 (3: 1: 1) and the other two were sterilized with the Sample 7 (1: 1: 3) and left for 10 minutes. Then swabs were taken from the skin after sterilization, and examined in the laboratory.

C- In Vivo Test

This test was accomplished at Istishari Medical Laboratory / Yarmouk / Baghdad. Istishari Medical Lab is a subsidiary of ASCO Group Holding and a joint venture with Iraqi Ministry of Health and Environment. In the Department of Medical and People's Clinics, positive swabs of coronavirus were collected from infected people and used in the PCR test.

The PCR test for COVID-19 is a molecular test that looks for genetic material (ribonucleic acid or RNA) of SARS-CoV-2, the virus that causes COVID-19 in a respiratory specimen. In PCR test, small amounts of RNA from specimens were intensified to create DNA. If the RNA contains signs of SARS-CoV-2, the replication process will continue until the virus is detected. Since February 2020, the PCR test has served as the accepted standard for diagnostics COVID-19 due to its precision and dependability [22].

Table 3. Different Samples Prepared in the Study

Sample	Constituents Ratio (PVP-I:H_2O_2:A.Vera)	pH	Povidone-Iodine volume (ml)	H_2O_2 volume (ml)	Aloe Vera volume (ml)
1	1:0.5:1	4.15	20	10	20
2	1:1:1	3.47	20	20	20
3	1:2:1	3.45	20	40	20
4	2:1:1	5.33	20	10	10
5	1:1:2	5.02	10	10	20
6	3:1:1	5.32	30	10	10
7	1:1:3	4.88	10	10	30
8	1:1.5:1	4.77	10	15	10
9	1.5:1:1.5	5.03	15	10	15

3- Results and Discussion

3.1. In Vitro Test

The In Vitro test was performed on the nine prepared samples as well as Dettol and an alcohol-based sanitizer for comparison. Fig. 1 shows the effect of varying Povidone-Iodine concentration on the effectiveness of the samples against bacteria. It was noticed that reducing Povidone-Iodine in the sample enhanced its efficacy, such as the points 10 and 15 ml volume which showed high kill rate (85-100%) for the four bacteria. This was in agreement with the findings of previous researches [6, 10]. PVP-I also showed high efficacy (100%), but not for all bacteria, at 30 ml volume. This result might be reached due to the synergistic effect of H_2O_2 [13]. Fig. 2 shows the effect of Aloe Vera concentration on the biocide effect of the samples. Increasing Aloe Vera extract concentration in the sample lowered its efficacy as for PVP-I. The sample showed high kill rate for all bacteria at 10 and 15 ml of Aloe Vera in the sample but it showed high kill rate not for all bacteria at 30 ml point. This was in agreement with the finding of a previous research [16] that Aloe Vera can be used as a complementary treatment alongside other methods. Fig. 3 clarifies the effect of changing hydrogen peroxide concentration on the effectiveness of the samples. It was clear that hydrogen peroxide was effective approximately at all concentrations studied in this research. The essence of hydrogen peroxide activity against microorganisms based on its capability of oxidizing these microorganisms and converting to oxygen and water. The oxidation process includes destroying the cell wall of these microorganisms. At 30 ml volume, the kill rate was 100% for all bacteria. At less or more than this volume, the kill rate was approximately 100% but not for all bacteria. It did not show the same efficacy on all bacteria because some kinds of bacteria have enzymes like lactoperoxidase (LP) which catalyzes the oxidation reaction producing a weak oxidizing agent of H_2O_2 which has bacteriostatic activity [23]. This was in agreement with other research [12]. For medical applications (3-9%) is recommended [2].

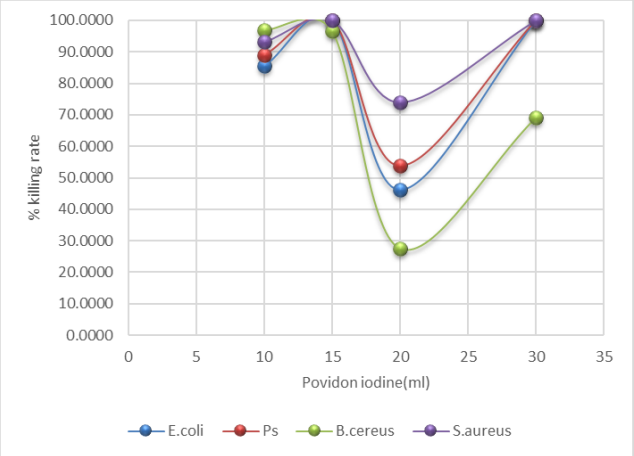


Fig. 1. The Effect of Different Amounts of Povidone-Iodine on the Effectiveness of the Samples Against the Four Types of Bacteria (In Vitro Test)

H_2O_2 activity is a time-dependent loss of viability. The concentration of H_2O_2 required to kill half the bacteria

within 15 s is 1.8 M (6%) but fall to 0.3 M (1%) at 2 min, to 10 mM (0.03%) at 1 h, and to 0.2 mM (0.0007%) with a 24-h exposure [23]. Nevertheless, it was noticed that hydrogen peroxide boosted the effectiveness of the sample at all volumes used. Although the effect of time on the sample was not studied in the present work but it could be reached to the following conclusion based on the literature. As mentioned later that H_2O_2 lose its efficacy with time as a result of continual dilution. This occurs when it exists in the solution alone. The existence of PVP-I with H_2O_2 in the solution resurge the efficacy by liberating molecular iodine continuously in the diluted solution as mentioned previously. The acidity function, pH of the prepared samples was within the acceptable range for an antiseptic (3-6) [6] i.e. in the acid range, therefore; its effect was not studied.

Fig. 4 shows the agar dishes after the application of these samples. The dishes were clear from the studied bacteria (S. aureus, E. coli, Ps. aeruginosa, and B. cereus). Fig. 5 shows a comparison among Sample 6, Dettol and alcoholic sanitizer against the studied bacteria. The sample was superior to Dettol and alcoholic sanitizer against all studied bacteria.

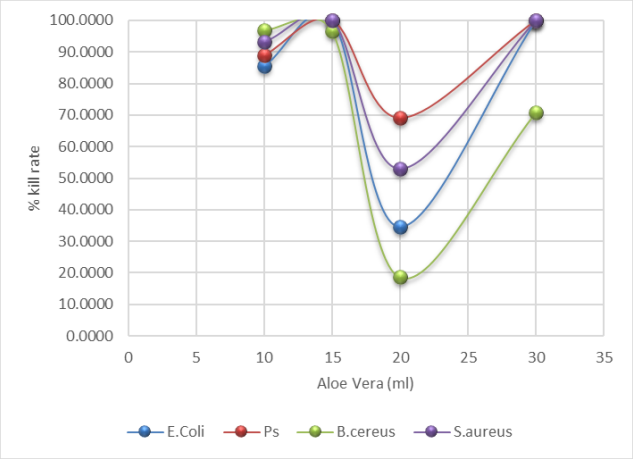


Fig. 2. The Effect of Different Amounts of Aloe Vera on the Effectiveness of the Samples Against the Four Types of Bacteria (In Vitro Test)

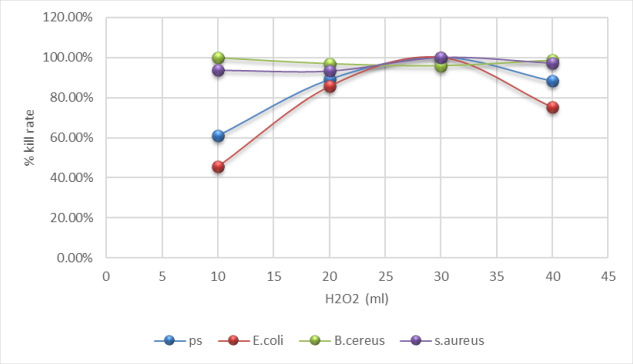


Fig. 3. The Effect of Different Amounts of Hydrogen Peroxide on the Effectiveness of the Samples Against the Four Types of Bacteria (In Vitro Test)

3.2. Ex Vivo Test

Samples 6 and 7 were further investigated by Ex Vivo Testing. First they were tested by visual observation on the skin of four mice after applying them on the mice's skin. It was observed that no skin sensitivity or redness occurred. The second step was done on the same mice by injuring them and contaminating the wound with two bacteria; *Staphylococcus aureus* and *Escherichia coli.* Two mice were contaminated with each bacteria. After 24 hours the wound became infected then the wound was sterilized with both samples. Finally, swabs were taken from the wounds, culturing and examining them in the laboratory. After applying the laboratory test on the samples, it was noticed that the agar dishes were completely empty from any bacteria as shown in Fig. 6 with each examination duplicated. This indicated that the prepared antiseptic had excellent effectiveness.

Fig. 4. Agar Dishes after In Vitro Test for Samples 6 and 7 in Addition to Alcoholic Sanitizer and Dettol. Samples Dishes Were Completely Empty from Bacteria

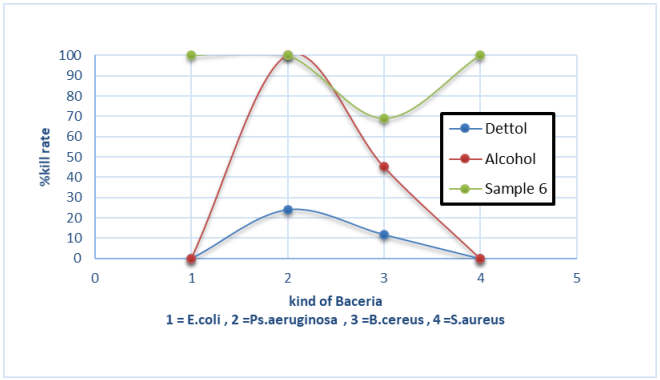
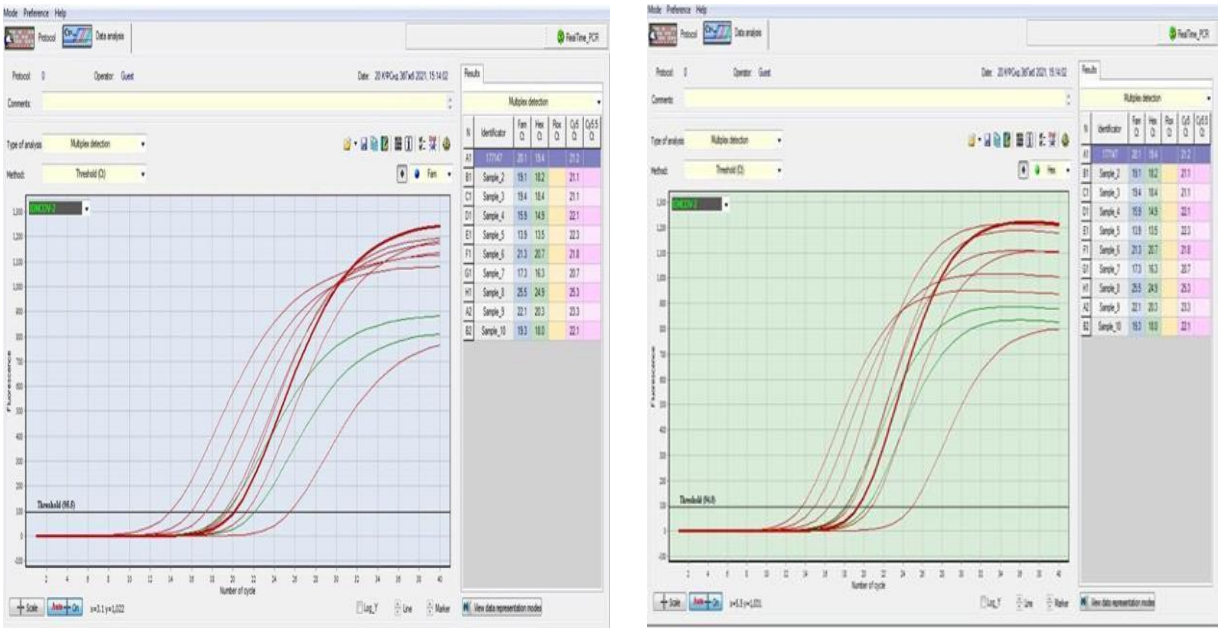


Fig. 5. Comparison between Sample 6 (3:1:1), Dettol and Alcoholic Sanitizer Applied on the Four Kinds of Bacteria (In Vitro Test)

3.3. In Vivo Test

For In Vivo test application on formulation samples 6 and 7, ten samples of positive swabs of COVID-19 were tested in PCR test. For every test there were two results for Hex and Fam wavelengths of fluorescence which represent y-axis. The thermal cycle represents the x-axis and the line parallel to the x-axis represents the point at which fluorescence is measurable. The existence of Corona RNA series was clear as shown in Fig. 7. Sample 6 and 7 showed ineffectiveness against COVID-19 virus. PCR test depended on extracting RNA from the virus cell and this was not compatible with the mechanism of iodine activity. One of the iodine mechanisms of action against microorganisms is by disrupting the cell wall of them leading to cytosol leakage [4]. and this cannot be detected by the PCR test.

Fig. 6. Agar Dishes after Ex Vivo Test of the Samples 6 and 7 Were Empty from Bacteria



(a) **(b)**

Fig. 7. PCR Test of (a) Hex Wavelength and (b) Fam Wavelength

4- Conclusions

A new antiseptic was prepared from PVP-I (10%), H_2O_2 (3%) and pure Aloe Vera in different ratios of these constituents depending on pH to be within the acceptable range for an antiseptic. The effect of each constituent was studied in detail and the results confirmed that each one of them was influential in the antiseptic's effectiveness. The new antiseptic was investigated by In Vitro test on four bacteria (Staphylococcus aureus and Bacillus cereus) as Gram-Positive type and (Escherichia coli and Pseudomonas aeruginosa as Gram-Negative. Four samples (samples 6, 7, 8 and 9) showed excellent efficacy in killing bacteria in comparison with alcoholic antiseptic and chloroxylenol (Dettol). These samples have the ratios of PVP-I: hydrogen peroxide: Aloe Vera: 3:1:1, 1:1:3, 1:1.5:1 and 1.5:1:1.5, respectively. Ex Vivo test was conducted on four lab mice using samples 6 and 7. The prepared samples showed an excellent efficacy in these tests. Also, the antiseptic was tested by PCR test against Coronavirus but the test could not demonstrate the effectiveness of the new antiseptic and another In Vivo test should be consulted.

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اختبار مطهر يد محتمل الاستخدام يتالف من بوفيدون اليود, بيروكسيد الهيدروجين والالوفيرا

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الخلاصة

في هذه الدراسة، تمت صياغة مطهر جديد واختباره لمطابقة فعاليته ضد الكائنات الحية الدقيقة. يتكون المستحضر من بوفيدون – يود (PVP–I) 10٪ (H_2O_2) 3% وهلام الصبار (نقي). تم تحضير نسب مختلفة من هذه المواد ضمن الحدود المسموح بها من pH بالنسبة لمطهر اليد (٣–٦). اختبرت النماذج التي تم تحضيرها. تم إجراء الاختبار في المختبر(In Vitro) باستخدام أربعة من البكتيريا، اثنان منها موجبة الجرام (Staphylococcus aureus) و (Bacillus cereus) واثنان سالبة الجرام (Escherichia coli) و (Pseudomonas aeruginosa) أظهر المطهر الجديد ١٠٠% نسبة قتل لكل من Escherichia coli، Pseudomonas aeruginosa و Staphylococcus aureus و٩٦,٤٦٦% نسبة قتل ل Bacillus cereus. عند مقارنة المطهر الجديد مع مطهرين شائعين (كلوروكسيلينول (ديتول) ومطهر يحتوي على الكحول) كان متفوقا لان المطهر الكحولي أظهر نسبة قتل ١٠٠% لكل من Escherichia coli، Pseudomonas aeruginosa و Staphylococcus aureus و ٨٩,٨٠٠٠% ل Bacillus cereus. لم يظهر الديتول نسبة قتل ضد البكنريا. تم إجراء اختبار خارج الجسم الحي(Ex Vivo) باستخدام العينة التي أظهرت أعلى فعالية في اختبار (In Vitro). تم إجراء ذلك عن طريق وضع المستحضر على جلد فئران التجارب بعد إصابة الجروح وتلويثها بنوعين من البكتيريا (Staphylococcus aureus) و Escherichia) coli). بعد وضع المطهر على الجروح، تم أخذ مسحات من الجروح لفحصها. أظهر المطهر الجديد فعالية مذهلة ضد البكتيريا بترك أطباق اكر خالية تماما من البكتريا. تم إجراء اختبار في الجسم الحي (In Vivo) أيضًا باستخدام اختبار تفاعل البلمرة المتسلسل (PCR) لـ COVID–19. المطهر الجديد لم يظهر فاعلية ضد فيروس كورونا لأنه لايمكن عزل الفايروس كما في البكتريا.

الكلمات الدالة: الصبار، المطهر، خارج الجسم الحي، نظافة اليدين، في المختبر، داخل الجسم الحي، بوفيدون – اليود.