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The Role of *Aerva Javanica* (Burm.F.) Juss. Growing in Jazan, Saudi Arabia as Antimicrobial and Coagulating Factor

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ABSTRACT

In deserted conditions, plants suffer from shortage of water and minerals. Though, they try to adapt with harsh conditions by building an internal defense system via making different chemical compounds, especially secondary metabolites. These secondary metabolites have several medicinal effect. Saudi Arabia is considered as semi-arid region. Therefore, it has many plants with high adaptation level to survive in the exotic environment. South west of Saudi Arabia is the richest in plant biodiversity. One of the common plants that grows nearly everywhere in the country is *Aerva javanica* (Burm.f.) Juss. It is a widely distributed herbal plant and is called desert cotton (in Arabic Tarf). It has different human usage. Flowers sometimes are used as cotton filling for pillows. Moreover, it has different medicinal practices among indigenous people. One of its uses among local people is using flowers to stop the bleeding if wounds persists. Therefore, this research investigate the medicinal implication of *A. javanica* as antibacterial agent and as a blood-coagulating factor. Dichloromethane, methanol, ethanol and water extractions from different parts of the plant (stem, leaf and flower) were investigated. The antimicrobial activity of plant extracts were evaluated with microorganism by disc diffusion method on three different strains of bacteria (*Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis)*. Moreover, coagulating rate by manual method on blood samples from female rat was restrained. *Escherichia coli* growth was inhibited only by the dichloromethane extraction from the three parts of the plant. *Staphylococcus aures* growth was inhibited by the aqueous extraction of the three parts of the plant and the methanol extraction of the leaves. All the other extractions has no effect on the growth of *Bacillus subtilis* or the other bacteria. For

the blood coagulating, all extracts were very effective on exceeding blood clotting time. The clotting time was less than the time of the control. The finding and results are discussed in detail.

Keyword: Aerva javanica, antibacterial, plant extract, medicinal plant, disc diffusion method, Saudi Arabia, blood coagulating.

INTRODUCTION

Plant extracts are mostly used medicinally to treat many diseases. Medicinal plants are found in different areas of Saudi Arabia. Several previous studies confirmed that Saudi Arabia has valuable medicinal plants and that environmental factors affected these plants positively (Sher & Alyemeni, 2011; Ali *et al.*, 2017).

Aerva javanica (Burm.f.) Juss. is a perennial herb belongs to the family Amaranthaceae, which comprises about 1000 species and 70 genera (Eshete *et al.*, 2016). It is a branching woody based herb 60 cm high with thick yellowish-green leaves; tassel with clustered white bud like flowers from the apex of the branching stem. A very widely distributed in Saudi Arabia.

A common use of this plant is that it used as fodder to goats, sheep, deer and donkeys (Qureshi & Bhatti, 2009). Traditionally, among the country's people, this plant has been used to treat many diseases, and it is therapeutic effect in alleviating liver disease which has been proved by Arbab *et al.* (2016). *Aerva javanica* contains tannin , which help in wound healing (Seifu, 2004). The powdered of *A. javanica* is applied to the ulcer externally in domestic animals (Chawla *et al.,* 2012). Also it is useful in stomach ache and bowel complaints, Skin infection, inflammation and abdominal worms (Ahmad, 2006). Moreover, it contains flavonoids component including kaempferol, chrysoeriol and isohemnetin-3-o-rutinoside. These compounds have anti-inflammatory properties (Saleh *et al.,* 1990). Extract of ethanol and water from *A. javanica* showed Antidiarrhoeal activity (Chawla *et al.*, 2012). Aerial part of *A. javanica* showed anti-diabetic and anti-oxidant potentials (Shad *et al.,* 2017). Furthermore, it is believed that this plant contains substances that act effectively as an antibacterial growth agent.

Consequently, in this research, we aim to show the activity of different extractions from different parts of *A. javanica* (stem, leaf, and flower) on three strains of bacteria: *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis.* Moreover, the effect of these extraction on blood clotting will be investigated, as the indigenous people of Jazan city use the flowers of the plant to stop wound bleeding.

MATERIAL AND METHOD

Plant collection

Samples were collected from Jazan city southern of Saudi Arabia in the spring of 2019. A specimen of the plant was pressed, prepared, labeled and deposited in the herbarium of King Abdulaziz University (KAUH, Jeddah). Plant was identified by author according to taxonomic procedure.

All samples were washed by tap water once to remove any particles or dust. Samples then were washed by distilled water and left to dry at room temperature for about two weeks until completely dry.

Dichloromethane extract

Samples were divided into stem, leaf and flower. Each part was crushed separately. Soxhlet device was used for extraction. According to the way of the device works, each thimble ,(Conical filter paper especially for this process), was filled with 2grams(g) of crushed sample, thimble was installed in the space reserved for the device. The empty tubes of the device were weighed. Seventy milliliter(ml) of chemical solvent (dichloromethan) was placed in the tubes of the machine, after running the device an hour and one third, the oil was extracted from the plant then the tube was weighted after extraction to measure the extracted oil ratio.

Ten ml of dichloromethan per tube was added and the extract was saved in refrigerator for later used.

Methanol extract

The same method used in the previous solvent but replaced with methanol was adapted.
Ten ml of methanol per tube was added and the sample was saved in refrigerator for later used.

Water extract

After leaving the parts of *A. javanica* to dry at room temperature they were split and grinded with nitrogen liquid to ensure plant drying.

10 grams of each part of *A. javanica* were placed with 100 ml of distilled water and placed on the heater until boiling (Al-Daihan *et al*., 2013).

Ethanol extract:

10 grams of grinded (stem, leaf and flower) with nitrogen liquid were macerated in 100 ml of solvent (ethanol) for 24h, with shaking for 12h at room temperature 25-30 degree (Al-Daihan *et al*., 2013).
All extracts were kept in refrigerator until use.

Antimicrobial assay

The microbe strains used in the study

Escherichia coli

Escherichia coli is a normal inhabitant bacterium of the intestines of most animals, including humans. Some strains of *E. coli* can cause a wide variety of intestinal, such as diarrhea, urinary tract infections, septicaemia, and neonatal meningitis (Ørskov & Ørskov, 1992).

Staphylococcus aureus

Bacteria in the genus *Staphylococcus* are pathogens for human and other mammals. The coagulase-negative staphylococci (CNS) are common commensals of skin, although some species can cause infections (Baron, 1996). *Staphylococcus aureus* produces toxins that can damage the host defense cell, these toxins are able to lyse erythrocytes of human and sheep (Lina *et al*., 1999).

Bacillus subtilis

some species of *Bacillus* have been increasingly implicated in a wide range of infections including abscesses, bacteraemia, septicaemia, wound and burn infections, endocarditis, osteomyelitis, respiratory, urinary tract infection and sophthalmitis (Baron, 1996).

Disc diffusion method

Antibacterial activity of different extract was carried out by disc diffusion method, where the discs are placed in the extracts to absorb the extract until saturated and then placed in petri dishes containing bacteria to test the effectiveness of the extract.

Antibiotic sensitivity testing

Antibiotic Multi-Test Discs have been used to ensure that the bacterial strains used are non-resistant.

Escherichia coli was inoculated in nutrient agar while *Staphylococcus aureus* and *Bacillus subtilis* were inoculated in blood agar. Then, the antibiotic multi-test discs were placed on the inoculated media.

The plates were incubated at 37°C for 24 hours (fig.1).

Antibacterial activity

The discs were immersed in the solute extract (dichloromethane, methanol and water) extracts from (stem, leaf and flower) for a week, to ensure disk saturation with the extract. Three plates were used for each bacterium. For each of the plates, a part of the plant (stem, leaf and flower) was placed and then the plates were divided into four sections (control, deichloromethane extract disc, methanol extract disc and the last disc of water extract). *Escherichia coli* was inoculated in nutrient agar, *Staphylococcus aureus* and *Bacillus subtilis* were inoculated in blood agar according to the Bergy standards. Then the discs were placed in the media (Nascimento *et al*., 2000).

The water extract from parts of the (stem, leaf, and flower) were also tested to absorb their effectiveness in inhibiting microbial growth without affecting their components by extraction. In addition, the powder part from *A. javanica* was tested (stem, leaf and flower) (fig.2).

The microbes were inoculated on the plate, and then the powder parts have been put without exposure to the extracts or dissolved.
All experiments were repeated three time (n=3) to confirm their validity.
All the plates were incubated at 37°C for 24 hours (fig.3).

Blood clotting

Female rat blood was drawn directly from the heart then it was used.
To determine the control, two ml of blood were put in empty tubes without any influencing factors and the clotting time was calculated. This method was repeated three times to confirm the approximate time of a normal clotting (fig.7).

Plant extracts and their effect on blood clotting

Each of the following extracts (methanol, water and ethanol) from all part of plant (stem, leaf and flower) were placed with three different concentrations (0.5 ml, 1 ml, 2 ml) in empty tubes, then two ml of blood was placed in the tubes containing the extracts and the clotting time was calculated immediately. Coagulation time was calculated by observing the appearance of thrombus (fig.8), while applying blood to the extract and tilting the tube (Houghton & Skari, 1994).

RESULTS

Antibiotic sensitivity test

Escherichia coli showed one inhibition zone around the discs of antibiotic where the diameter of the inhibition zone was measured from three directions and the average length of the diameters of inhibition zone was 2.3cm, while *S. aureus* showed one inhibition zone and the average length of the three diameters was 1.6cm. The last strain *B. subtilis* showed two-inhibition zone around antibiotic discs with differentiation in the size of these inhibition zone. The average diameters of the first zone of inhibition were 2.6cm and 1.7cm. In general, it could be said that all strains showed no resistance against antibiotics (fig.1).

Antimicrobial activity

After antibiotic sensitivity test and making sure that no bacterial strains were resistant to antibiotics, the experiments showed the following:

Escherichia coli

Dichloromethane extract from (stem, leaf and flower) showed inhibition zone in the growth of *E. coli.* Where the average of inhibition diameters of the stem and leaf extract was 0.5cm, in flower part it was 0.4cm (fig.4). On the other hand, no inhabitation zone was recorded in the aqueous and methanol extracts on *E. coli.*

Staphylococcus aureus

The aqueous extract from all parts of the plant (stem, leaf and flower) and the methanol extract from the leaf part showed inhabitation zone in *S. aureus.* The average of diameter Inhibition zone from aqueous extract of stem and leaf were 1.6cm, while in flower part it was 1.3cm. The average of diameter Inhibition zone from methanol extract from leaf part only was 0.7cm (fig.5).

Bacillus subtilis

All the extractions from stem, leaf and flower showed no inhibition zone in *B. subtilis* (fig.6).
In addition, the powdered plant parts without extracting showed no effect on the microbial growth as shown in (fig.2) and (table.1).

Blood clotting

The average time of coagulation in the control sample was 55.3 seconds.
All extracts showed an effective result on blood clotting. When applying the extracts, the clotting time was faster than the control in all samples. Some of the extracts had instantaneous results as soon as the blood was applied to the extract. Table.(2) shows the clotting time for each of the extractions with different concentrations of plant parts (stem, leaf, and flower).

Stem

The ethanol extract from the stem showed faster clotting time(0sec.) than methanol(4-2sec.), then the aqueous extract(6-2.5sec.).

The 2ml of concentration from all extracts in the stem part showed faster blood clotting, followed by 1ml then 0.5 ml.

Leaf

The methanol extract showed faster blood clotting time(0sec.) followed by ethanol extract(6-0sec.). The slowest was the aqueous extract (39-17sec.).

2ml concentration of all extracts gave the fastest clotting time, followed by 1ml. and 0.5ml was the slowest.

Flower

The aqueous extract showed faster blood clotting time (3-1.5sec), followed by ethanol extract (10-5sec.) then methanol extract was the slowest (23-20sec.)

2ml concentration of all extracts of flower part showed the fastest blood clotting (20-1sec.), followed by 1 ml. while 0.5ml concentration was the slowest.

Comparing the extracts used from the different plant parts of plant

The methanol extract from the leaf showed faster clotting time than the stem followed by the flower.

As for **the aqueous extract**, the clotting time from the flower part was faster, then stem and leaf.

For **the ethanol extract**, the stem part extraction was faster in clotting then the leaf and the flower.

In general, all extracts showed their effectiveness on blood clotting and the results of clotting were faster than the control. Consequently, there is a positive relation between the concentration and the time for coagulation. The highest the concentration is, the less time for coagulating.

DISCUSSION

In the current research, the medicinal important of *Aerva javanica* as antimicrobial and blood coagulating factor was investigated. Three solvent extractions: Aqueous, dichloromethane and methanol were used. Moreover, powdered of the grounded plant was used as crude with no solvent added. All plant parts were used separately (stem, leaf and flowers). The results showed an effect of the dichloromethane extract of the all three parts of the plant on *E. cloi* which could be seen by a zone inhibition around the bacteria. This result is in agreement with Shad *et al*. (2016).

Several previous studies have shown a positive effect of the extracts of *A. javanica* on microbial growth. In a study by Mufti *et al*. (2012) the aqueous extract and the crude fraction of *A. javanica* showed inhibition against the growth of *E. coli* and *S. aureus*. The results of aqueous extract on *S. aureus* is in agreement with the current study but it is in disagreement with *E.coli.* Also the result of crude fraction effect on both *E.coli* and *S. aureus* does not agree with present research, since the powdered crude extract of the plant (stem, leaf, and flower) showed no effect on the inhibition zone growth of the three strains of bacteria used.

The methanol extract of the leaf had a positive effect in inhibiting the growth zone of *S. aureus*, this result is in agreement with the findings of Al-Fatimi *et al*, (2007) and Shad *et al*. (2016).

The aqueous extract of *A. javanica* of the all three parts of the plant has no effect of zone growth inhibition on *E. coli*, *S. aureus* or *B. subtillis*. This supported the previous result found by Abutbul *et al*. (2005) and Al-Fatimi *et al.* (2007).

The differences in the effect of inhibition of the growth zone between the findings of the existing study and other studies could be referred to the plant extraction solution which gives different compound according to the solvents used.

Blood clotting effect of the extracts

In general, all extractions from all parts of the plant had faster clotting time than the control. When comparing the three extract solvents, the ethanol extract from the stem and the methanol extract from the leaves were the fastest in blood clotting with zero seconds time. On the other hand the slowest extract solvent was the aqueous extract from the leaves with time 39-16sec. This could prove that the secondary metabolites which are extracted by either methanol or ethanol are the factors of coagulating effect.

Comparing the parts, the stem extraction with all solvents was the fastest followed by the flower extractions and finally the leaf extractions had the slowest time.

The highest the concentration of the extraction, the fastest the clotting time.

This coagulating reaction could be referred to the presence of tannins in the tissues of *A. javanica* as detected by Seifu (2004). As far to our knowledge this is the first study that concern about the effect of *A. javanica* extraction on blood clotting. Previous studies have been done such as (Kathiresan *et al*., 2006) and (Adhyapak & Kachole, 2016) on other plant extracts but not *A*. *javanica*. The result of those studies reported the coagulating reaction of some plant extracts on blood and recommended the cautious consume of herbal medicine.

CONCLUSION

This study was carried out to investigate the effect of the plant *Aerva javanica* as a blood coagulating factor and as antimicrobial agent. Three types of extracts were prepared in addition to the crude powdered plant. Three species of bacteria were used to test the antimicrobial effect.

The powdered plant has no antimicrobial effect which means that the effective substance should be extracted first to have an influence on the microbes.

Leave extraction was the most effective regardless the extraction solution. This could be due to that leaves are the place were secondary metabolites are performed.

Both *Escherichia coli* and *Staphylococcus aureus* growth have been inhibited by the extractions with different response degrees, while *Bacillus subtilis* was resistance to all extracts.

Aerva javanica extractions from all parts of the plant, and the crude grounded plant showed a positive effect on blood clotting. The clotting time of the extracts was less than the clotting time of the control. This approves the theory of this research which was adapted from the indigenous people who use the flowers of *A. javanica* to stop wound bleeding.

On conclusion, *Aerva javanica* can be used as a blood-coagulating and as antibacterial agent. However, this plant should be carefully consumed. More studies should be done on the chemical constituents of *A. javanica* to emphasize the effective chemical substance in each part of the plant.

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Table 1: antibacterial activity. S: stem, L: leaf, F: flower, (-) no inhibition zone

Test organisms / **Plant extract**	**Plant Part**	***Zone of inhabitation*** **In cm**		
		S. aureus	***E. coli***	***B. subtilis***
methanol	Stem	-	-	-
	Leaf	0.7	-	-
	Flower	-	-	-
dichloromethane	Stem	-	0.5	-
	Leaf	-	0.5	-
	Flower	-	0.4	-
Water	Stem	1.6	-	-
	Leaf	1.6	-	-
	Flower	1.3	-	-
Crude of plant	Stem	-	-	-
	Leaf	-	-	-
	Flower	-	-	-

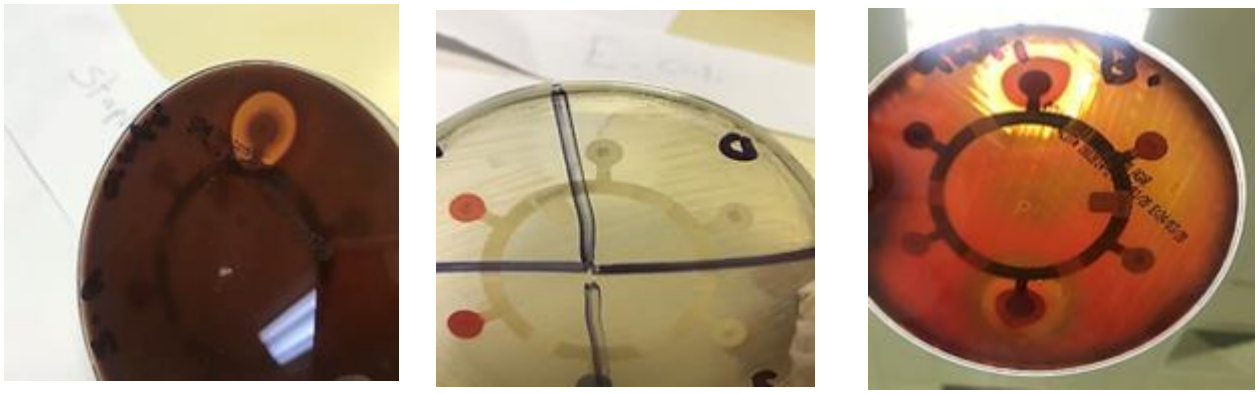


Figure 1: Antibiotic sensitivity test

S.aureus *E.coli* *B.subtilis*

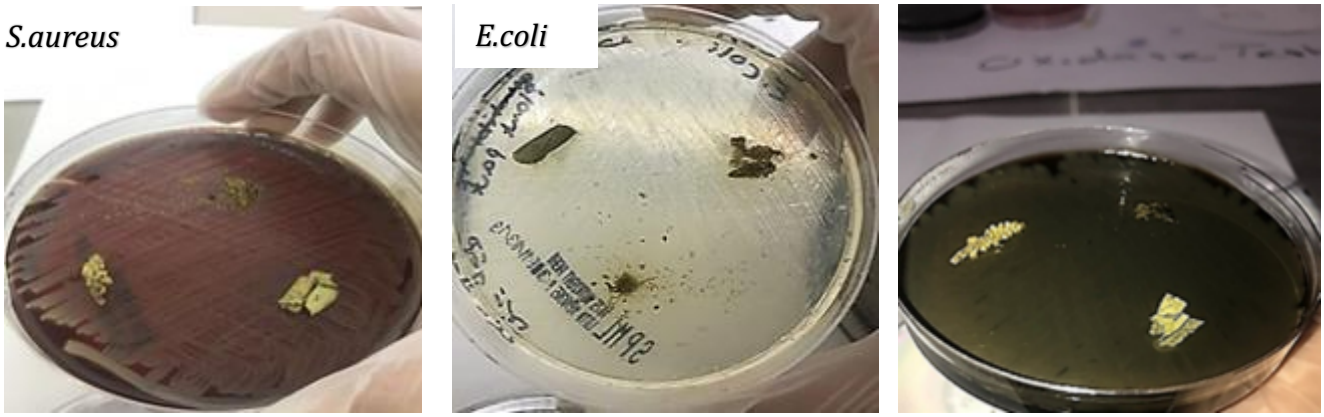


Figure 2: Plant part without extraction

S.aureus *E.coli* ***B.subti***

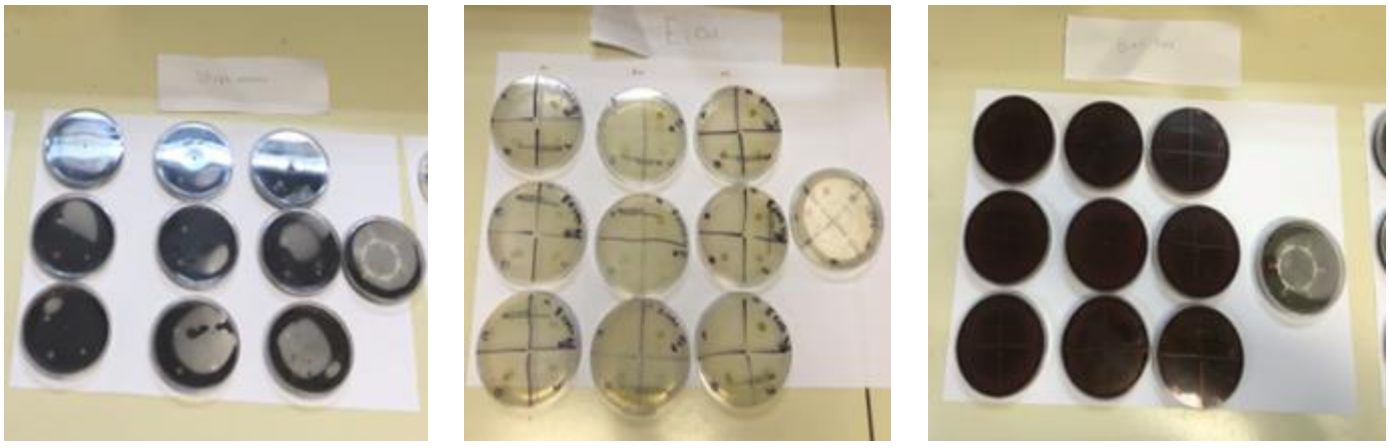


Figure 3: Antibacterial activity test

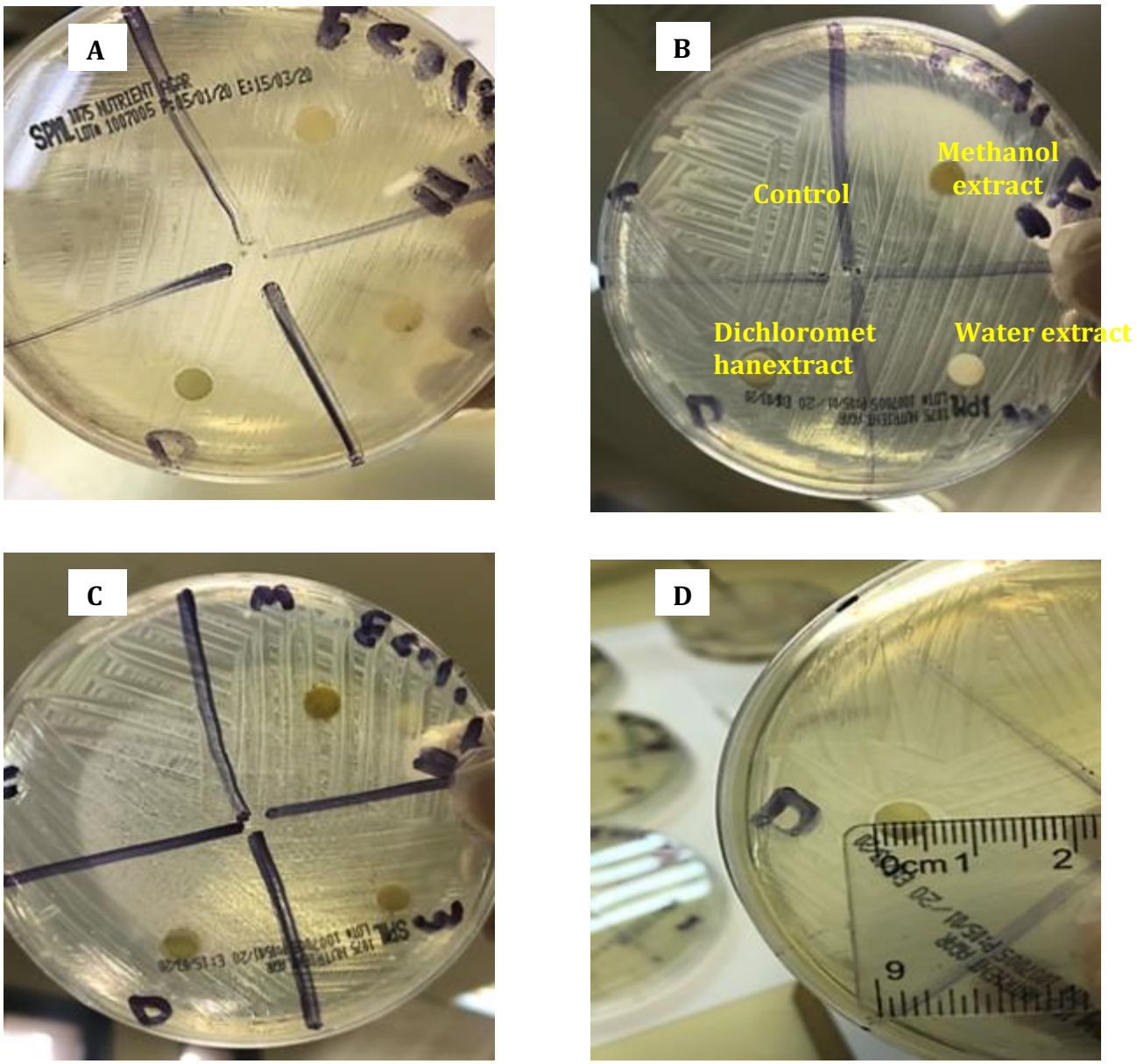


Figure 4: Antibacterial test of E.coli; A: extract from stem, B: extract from flower, C and d extract from leaf

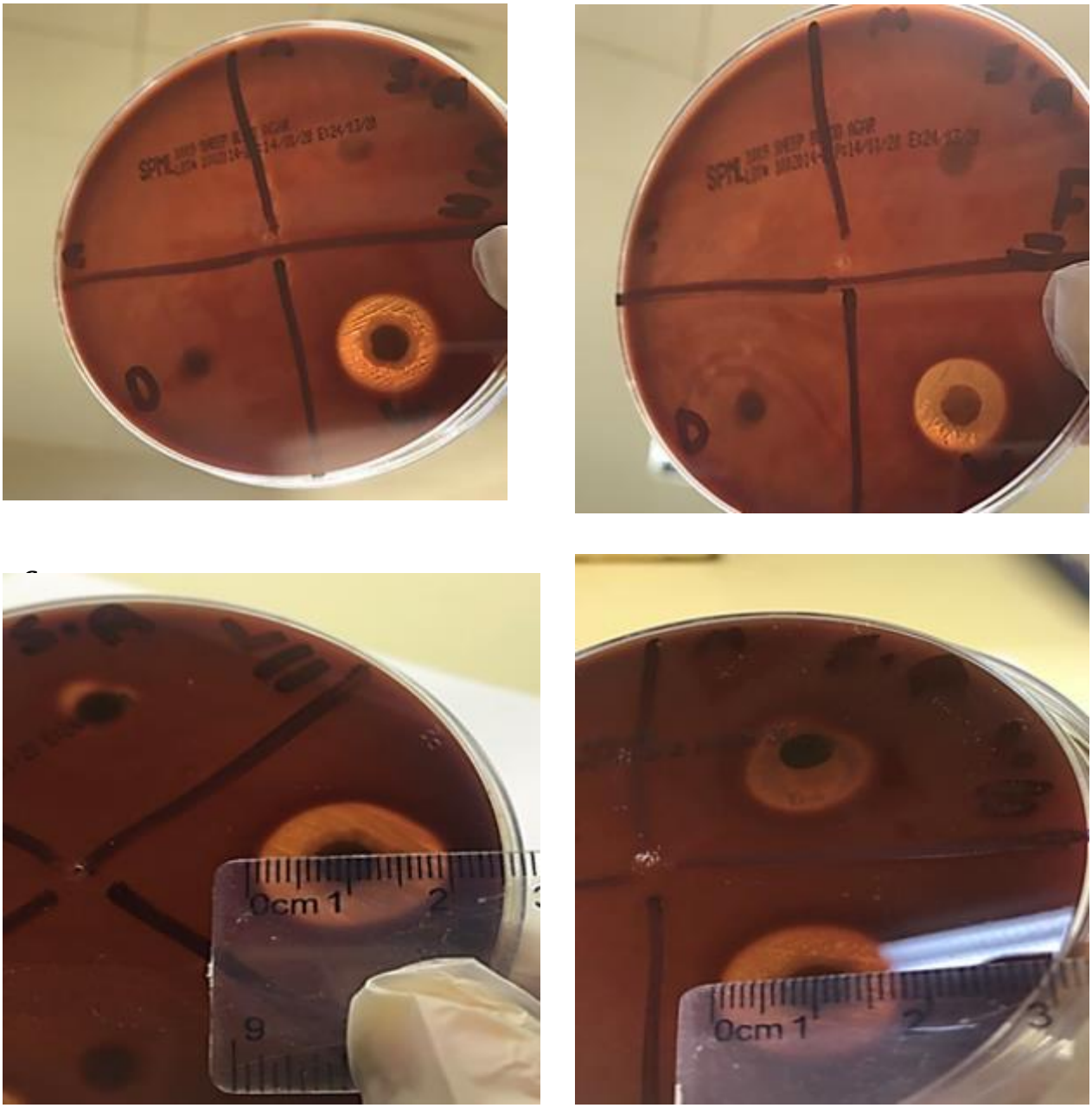


Figure 5: Aibacterial test of S.aureus ; A:extract from stem , B:extract from flower, C and D extract from leaf

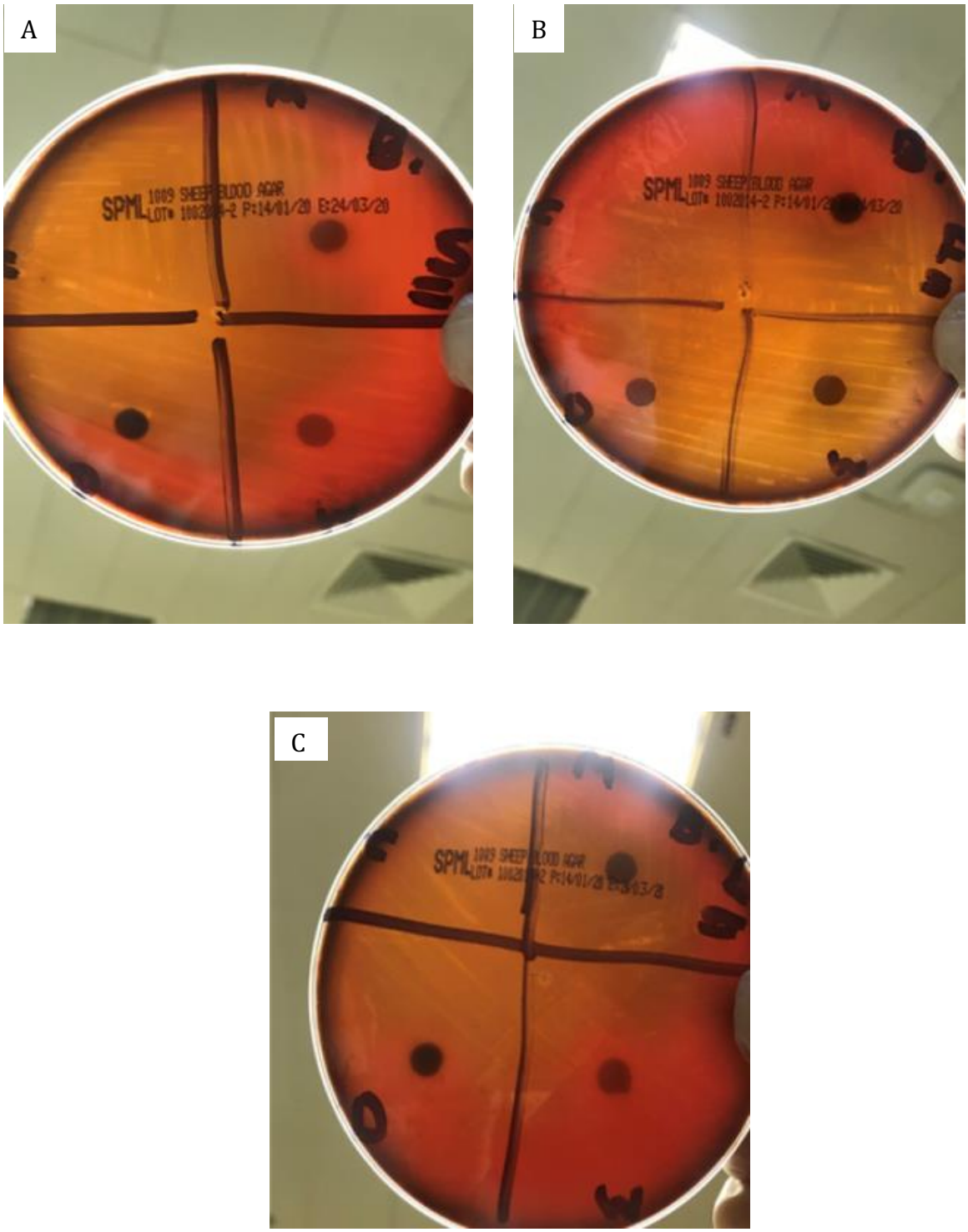


Figure 6: Antibacterial test of B.subtilis; A: extract from stem, B: extract from flower, C: extract from leaf

Table 2 :Plant extract and effecting of coagulation time _ with 2ml of blood

Plant extract concentration	Stem			Leave			Flower			Time of clotting with 2 ml of blood (control)	
	Methanol	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Water	Ethanol		
0.5 ml	4:26s	6:65s	The moment it touched	The moment it touched	35:83s	5:62s	23s	2:80s	10s	First sample	1 minute
1ml	1:21s	3:48s			23:25s	6:13s	24s	1-5s	7s	Second sample	1.02 minutes
2ml	1:52s	2:46s			16:65s	The moment it touched	20s	1-5s	5s	Third sample	50 seconds

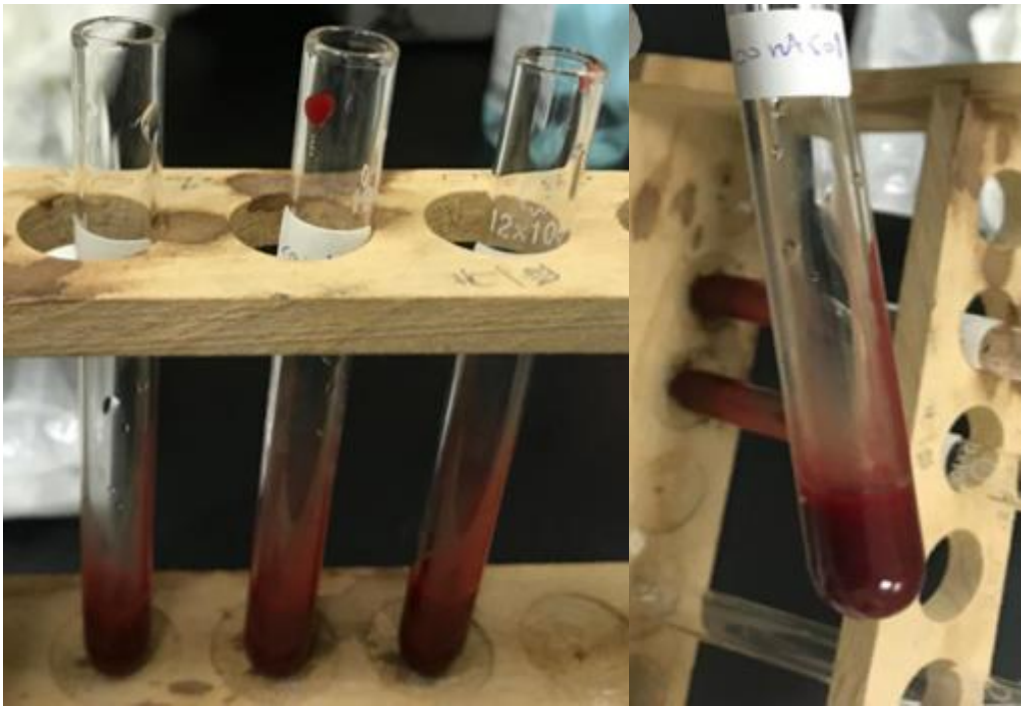


Figure 7: Blood without extract

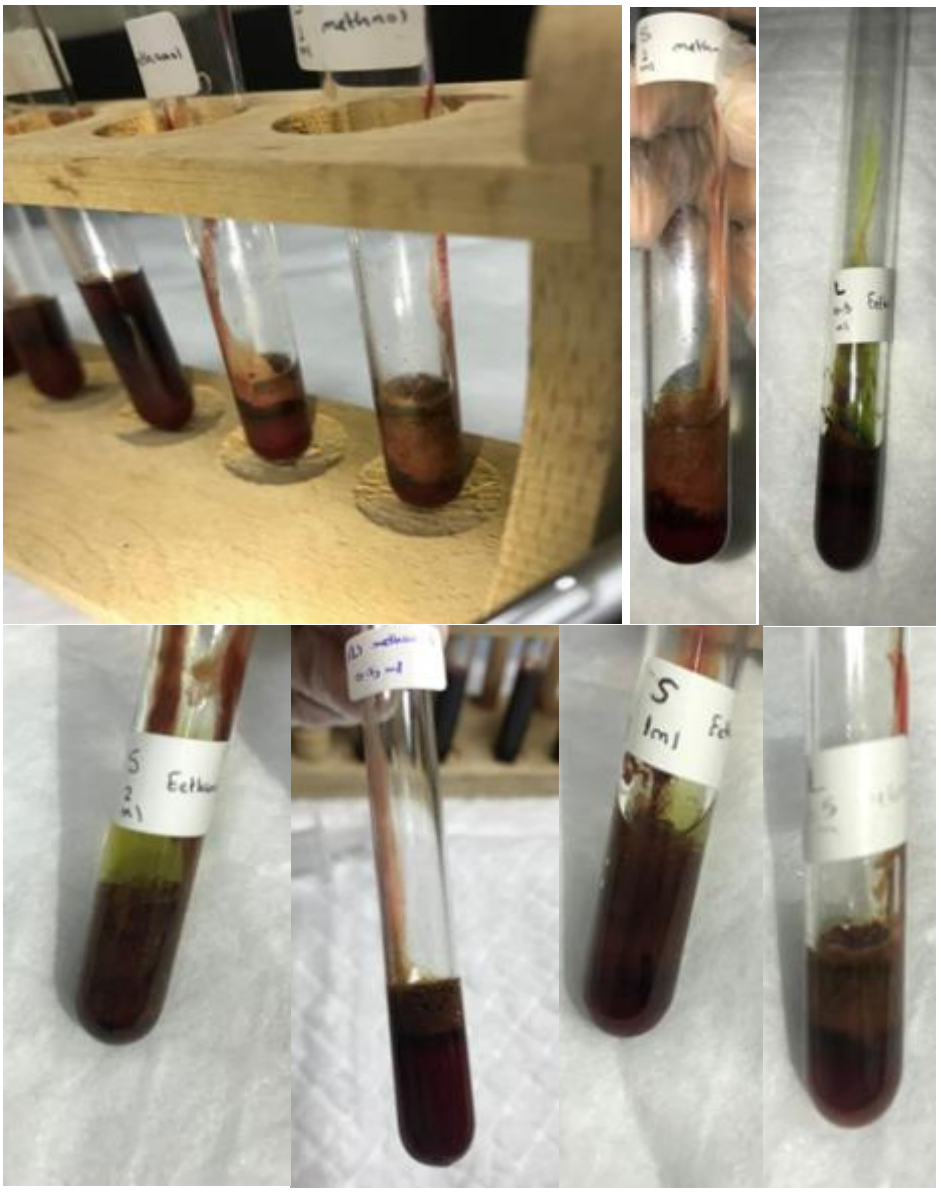


Figure 8: Blood samples with extractions.