

Effect of some medicinal plant extracts on pathogenic bacteria and fungi in Albaydha governorate, Yemen

Hamid mohammed Al-gabr^{1*}, Amin M. Alwaseai², Mohammed alfakih¹, Huining, Zhang³, Rasel mukred¹, A. Gashaa¹, T Alezani¹, K. Almaameri¹, K. Hassen¹, M. Almodhafery¹, M. Alfaydey¹, N. Jeraan¹.

Corresponding author. Hamid mohammed Al-gabr. biology department, education college, Albaydha University. Yemen. han-chin@hotmail.com

Amin mohammed alwaseai. Biotechnology and Food Technology department, Faculty of Agriculture, Tamar University, Yemen. amin_alwaseai@yahoo.com

Mohammed ali alfakih, Biology department, Education college, Albaydha University, Yemen fakih2005@hotmail.com.

Huining Zhang. NingboTech University, Ningbo 315000, China. zhanghn@nbt.edu.cn

Rasel ahmed mukred. . biology department, education college, Albaydha University. Yemen. rasel.mukred@gmail.com

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Abstract

The medicinal plants which are used in traditional medicine for treating of many diseases. These plants contain many materials as alkaloids, flavonoids, glycosides etc. that make them effective for use in medical purposes. Seven plant extracts (Black seed, Rue, Radish, Onion, Cactus, and Guava) were used in this study. The paper disc diffusion assay method was used in this experiment. The test started by inoculating a nutrient agar dish with 1ml of bacterial cultures. Bacteria genus (Staphylococcus aureus, Escherichia coli) were obtained from patient's ear swab in Alqysi Hospital, Albaydha. The fungi Aspergillus niger were also obtained from ear swabs samples by using Sabouraud Dextrose Agar (SDA). Three replicates were prepared for each extract in this study.

The results indicate that the most effect against the microbes were Origanum syriacum, Vigna unguiculata, Zizphus spina-christi, and Psidium guajava. The other medicinal plants have no high disinfect. Also the results show that the Rumex nervosus was disinfected against A. niger and staphylococcus aureus, but have no action against E. coli. The plant extract Zizphus spina-christi shows moderate effect which is inhibition of the fungi A. niger. In contrast, the Psidium guajava shows high efficiency against E. coli and is not effective against the other microorganism under study.

The study is the first in Yemen that investigated the effect of the mentioned medicinal plants against the bacteria Staphylococcus aureus, and E. coli. It came up with good results for the inhibition of fungi.

Keywords: Medical Plant, Bacteria, Fungi, A. niger, Staphylococcus aureus

المخلص

النباتات الطبية والعطرية تستخدم لمعالجة العديد من الامراض بطرق شعبية. لان هذه النباتات تحتوي على مواد فعالة تعطيتها القدرة على مكافحة الامراض و استخدامها طبيا مثل الجليكونات ، الفلويديات .. الخ.

تم استخدام سبعة نباتات طبية مثل (الحبة السوداء، السذاب، الفجل، البصل، الصبار، والجوافة) تم استخدام طريقة الأقراص المشبعة بمستخلص النبات. حيث تم استخدام بيئة الاجار المغذي الملقح بالميكروب بإضافة (1مل) من معلق البكتريا (Staphylococcus aureus, Escherichia coli) والتي تم الحصول عليها من مسحات الاذن من المرضى في مستشفى القيسي، مدينة البيضاء. الفطر (Aspergillus niger) أيضا تم الحصول عليه من مسح عينات الاذن وتم تنميته في بيئة الدكستروز المغذي. تم عمل ثلاث مكررات لكل عينة. اشارت نتائج الدراسة الى تأثير فعال لمستخلص النباتات (Origanum syriacum, Vigna unguiculata, Zizphus spina-christi, and Psidium guajava) ومستخلص النباتات الأخرى لم يكن لها تأثير واضح. أيضا مستخلص النبات (Rumex nervosus) كان له فعالية ضد الفطر (Aspergillus niger) والبكتريا (Staphylococcus aureus)، لكن لم يحدث أي تأثير على بكتريا (Escherichia coli). ولوحظ تأثير متوسط لمستخلص النبات (Zizphus spina-christi) على الفطر (A. niger)، بينما كان (Psidium guajava) ذو تأثير تثبيطي عالي ضد البكتريا (E. coli) ولم يظهر أي تأثير ضد الميكروبات الأخرى تحت الدراسة. كانت هذه اول دراسة في اليمن تدرس تأثير هذه المستخلصات النباتية ضد البكتريا (Staphylococcus aureus, E. coli) والفطر (A. niger)

الكلمات المفتاحية: النباتات الطبية، البكتريا، الفطريات ، Staphylococcus aureus

Introduction

Recently, several studies have investigated the effect of some medical plants for the inhibition of bacteria and fungi which cause many diseases for humans and animals. Using a medical plant as a drug since many decades is known as traditional treatment. Many people in Yemen prefer using medical plants and the culture of the country encourages using this treatment as it is a safe treatment and sometimes gives a good result. Consequently, the researchers have tried to investigate the properties of the medical plants which are generally used in traditional treatment. (Thembo et al., 2010) investigated the antifungal activity of plant species viz. *Tagetes minuta*, *Lippia javanica*, *Amaranthus spinosus* and *Vigna unguiculata* against isolates of fungi, i.e. *Fusarium verticillioides*, *F. proliferatum*, *Aspergillus flavus* and *A. parasiticus*. The results indicated that all extracts, except for the water extracts, showed growth inhibitory activity against most isolates of the *Fusarium spp.* while in the methanol and hexane extracts, no inhibition of the *Aspergillus spp.* tested was observed. Furthermore, (Haq et al., 2020) and (yaseen et al., 2020) tested antifungal properties of *Aloe vera* plant against bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus Subtilis*, *Acinetobacter baumannii*, *Bacillus cereus* and *Enterococcus*. The results showed the high inhibition appears on *Escherichia coli*. In the same field, (Dalhat et al., 2020) studied the extracts of leaves of plant species such as *Momordica charantia*, *Prosopis juliflora*, *Ocimum sanctum* and *Mitracarpus scaber* and (Irowa et al., 2020), used the extracts of *Cerathoteca sesamoides* and *Chromolaena odorata* for their antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and

klebsiella pneumoniae and the fungi as *Candida albicans* (Abubakar et al., 2020 and Omojoyegbe et al., 2020) investigated extract of *M. charantia* and *Chrysophyllum albidum* and showed high degree of activity followed by *P. juliflora* and *O. sanctum*, against some bacterial isolates except *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. In addition, (Kamal et al., 2020 and Coker and Oaikhen, 2020) used the leaves of *Ficus carica* Linn and *Ficus thonningii* against some human pathogenic bacteria isolated from Urinary tract infections. The *Candida* isolates from vagina ((Coker and Oaikhen, 2020) and the results indicated *Ficus carica* L. and *Ficus thonningii* regard a good source for controlling human pathogenic bacteria isolated from Urinary tract infections. On other hand the study of (Eman and Fatimah, 2020) used *Ziziphus* and *Rumex* extract. In vivo, results showed that both *Ziziphus* and *Rumex* aqueous extracts had positive synergistic. While (Geta and Kibret, 2020) evaluated *Acanthus sennii* against pathogenic bacteria. and indicated high antibacterial activity against strains of *Staphylococcus aureus*, (Joshi et al., 2020) and Njokuocha and Ewenike, 2020) used extract plant leaves (*Moringa oleifera*) against *staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results indicated antibacterial activities against all the bacterial isolates. The (Bulugahapitiya et al., 2020) used the leaves *Dialium thwaites* and studied them against strains of bacteria; *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Methicillin Resistant *Staphylococcus aureus* and fungus; *Candida albicans* were used as test organisms. The results showed high activity against *Staphylococcus aureus* (Gram Positive). In addition, the study of (Njokuocha and Ewenike, 2020) used *Pterocarpus santalinoides* L'Herit DC and *Ceiba pentandra* L extract and

noted that *Pterocarpus santalinoides* showed inhibitory activity only on *Salmonella typhi* and *Escherichia coli*. The *Ceiba pentandra* showed activity against all the bacterial isolates except *Salmonella typhi*. *E. coli* as the most susceptible to the leaf extracts. *Salmonella typhi* was not sensitive to the leaf extracts of *Ceiba pentandra*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were not sensitive to the leaf extracts of *Pterocarpus santalinoides*. (Enejiyon et al., 2020) investigated activities of *Allium sativum* and *Allium cepa* against *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Escherichia coli*. The antibacterial activities showed maximum activities against *S. aureus*, *S. pneumoniae* and *S. aureus*. (Fabowale et al., 2020) investigated the antifungal and antibacterial activities of extracts of *Trema orientalis* linn. Blume. against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Streptococcus faecalis*, *Shigella dysenteriae*, *Proteus mirabilis*, Haemolytic *Streptococcus viridian*, *Aspergillus niger*, *Candida albicans*, and *Aspergillus flavus*. The antibacterial assay showed the efficacy of the methanol and petroleum ether extracts except on *S. aureus* ATCC 43300 and *Shigella dysenteriae*. In their study (Madasamy et al., 2020) studied the extract *Caralluma adscendens* var. *attenuata* (MECA) and their antimicrobial activity was tested against various clinical pathogens, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, and *candida albicans*. The 200 mg FCA was active against *Escherichia coli*, *Staphylococcus aureus*, *E. faecium*, and *C. albicans* of highly significantly difference than FCA 50 mg and FAC 100 mg. the study of (Tariq et al., 2021) investigated the effect of *Verbascum arianthum* as anti-microbial activity. The observed results showed highly significant antifungal activity against

Aspergillus Niger. In some studies which the nanotechnology was used to give the plants extract more efficacy against microbial like the study of (Elemike et al., 2020) which used Silver nanoparticles with extracts of Welsh onion plant. The photocatalytic property increased with increase in the concentration of the precursor salt (AgNO₃) from 1 to 5 mM. Gram positive *Staphylococcus aureus* and *Bacillus cereus* and Gram negative *Klebsiella pneumoniae* and *Escherichia coli* bacteria strains were susceptible to the silver nanoparticles (2 mM). The nanoparticles were most active against *E. coli*. On the other hand some fungi could produce mycotoxins which affect directly humans and causes several diseases (Al-gabr et al. 2014). The study of (Nasir and Ali, 2020) was carried out to isolate and identify fungal species producing mycotoxins from dried figs using Cinnamon extract. The results showed that different concentrations of Cinnamon extract had high efficacy in inhibition of *A. niger* where the inhibition was increased with increasing Cinnamon extract concentration. (Islam et al., 2015) conducted a study on efficacy of extracts of garlic clove, allamanda leaf, neem leaf and marigold leaf against some fungi as *Bipolaris sorokiniana*, *Ahernaria tenuis*, *Curvularia lunata*, *Fusarium* spp, *Aspergillus niger* and *Aspergillus flavus*.

(Faharan et al., 2008) used in their study the extract of the plant guava (*Psidium guajava*) against *E. coli*, and *Staphylococcus aureus* and the results showed high efficiency against (*E. coli*), while the bacteria (*S. aureus*) showed no effect.

Hence, this study aimed to investigate the effect of some medicinal plant extracts which are used in my country (Yemen) to treat this bacteria and fungi which cause some diseases in ear and skin infections.

Materials and Methods:

1- Medical plant collection:

Seven medical plants were chosen according to their use in Albaydha Governorate in traditional medicine for treatment in some diseases. *Aloe vera*, *Origanum syriacum*, *Punica granatum*, *Vigna unguiculata*, *Zizphus spina-Christi*, *Psidium guajava* and *Rumex nervosus*, were collected fresh and transferred to the laboratory to be used in this study.

2- Chemicals and equipment:

Media culture used in this study were **Blood Agar** (Meat extract 10g, Tryptone 10g, Sodium chloride 5g, Agar 15g, Final pH 7.3 ± 0.2), **MacConkey Agar** (Peptone 20g, Lactose 10g, Bile Salts #3 1.5g, Sodium chloride 5g, Neutral red 0.030g, Crystal violet 0.001g, Agar 15g, Final pH 7.2 ± 0.2), and **Mueller Hinton agar** (Peptone 17.5g, Beef infusion solids 2g, Starch 1.5 g, Agar 17g, Final pH 7.3 ± 0.2), for isolation and identification of bacteria. For isolation and identification of fungi the media culture used is Sabouraud dextrose agar (SDA) (D(+)) Glucose 40g, Casein peptone 5g, Meat peptone 5g, Agar 15g, Final pH 5.6 ± 0.2).

3. Extracting plant samples:

According to the ((Al -Naser and Ezz Al-dden, 2014), (Mohana and Raveesha, 2007), (Webster et al., 2008), (Lee et al., 2007)) methods, the leaves samples were washed with distilled water carefully and allowed to dry in room temperature until dried. After that, the dried leaves were pulverized and the powder plant were separately divided into three portions as 1, 2, and 3 grams, and were macerated with 50 ml hot distilled water, and mixed by a blender for 5 minutes. The different extracts were filtered separately off through a cotton plug and finally with a Whatman No. 1 filter paper, and centrifuged for 15 minutes with high speed 5000 cycle/ minute. The liquid filtrates were concentrated and evaporated to dryness at oven under 50C temperature, and each extract was transferred into well-labeled sterile glass vials

and stored at 4 °C until using (Okokon et al., 2012).

4- Bacteria isolation:

Tow bacteria genus (*staphylococcus aureus*, *Escherichia coli*) were obtained from patients' ear swab in Alqysi Hospital, Albaydha by using different media culture for the identification of bacteria and the general serology test.

5- Fungi isolation:

The fungi *Aspergillus niger* was also obtained from ear swab samples by using Sabouraud Dextrose Agar (SDA). This fungus was identified by microbiology lab. At Alqaisi Hospital. The isolation was harvested after incubation for 3-7 days and purification by using one spore in new media culture for further study. For identification, the fungi was sub-cultured on (SDA) and Czapek media, and phenotypically identified on macroscopic and microscopic characteristics were identified under light microscopy (Pitt and Hocking, 2009).

6- Effectiveness of plant extract on bacteria:

The method used for this experiment was the paper disc diffusion assay. The test started by inoculating a nutrient agar dish with 1ml of bacterial cultures. Fifty microliters containing 1,2,3 mg/L of each herb extract were absorbed by the paper disc (8mm in diameter) placed on the top of agar plate. The plates were incubated at 37 °C for 24 h and the antimicrobial activities were assessed based on measurement of the diameter of the clear zone around the paper disc. Three replicates were prepared for each extract in this study (Lee et al., 2007).

7- Effectiveness of plant extract against fungi (*A. niger*)

According to the method (Shamsi et al., 2016), 15 ml of autoclaved SDA medium supplemented with 1 ml plant extract in different concentration (1,2,3 mg/L were separately poured into Petri plates from test tubes and allowed to cool and solidify. The Petri

dishes containing medium were devoid of the extract but with same amount of distilled water served as control. After the completion of solidification of the medium, 5 mm disc of seven-day old culture *A. niger* was inoculated in the center of the Petri dishes with solidified SDA medium. The plates were incubated at $25 \pm 2^\circ\text{C}$ for seven days. After incubation, the colony diameter was measured in mm. For each treatment three replications were maintained.

8- statistical analysis

Analysis of variance was applied for all treatments using the SPSS software program, version 24 IPM Corp. means of the medicinal plants were compared using one-way ANOVA analysis and T-test ($p \leq 0.05$). The correlation

between the concentration of plant extracts was determined.

Results:

Effect of plant extract on the inhibitor of bacteria:

The result showed in table (1, 2) and figures (1) present the effect of medical plant extract against bacteria *S. aureus*, and *E. coli* the concentrate 3mg/L to be more effective than the other concentrations, which showed the *Origanum syriacum*, *Zizphus spina-christi*, *Aloe vera*, and *Vigna unguiculata* the diameters are 22,20,18,18 mm respectively. The results indicate that the plant extracts *P. granatum*, and *Psidium guajava* have no effect inhibition against bacteria *S. aureus*.

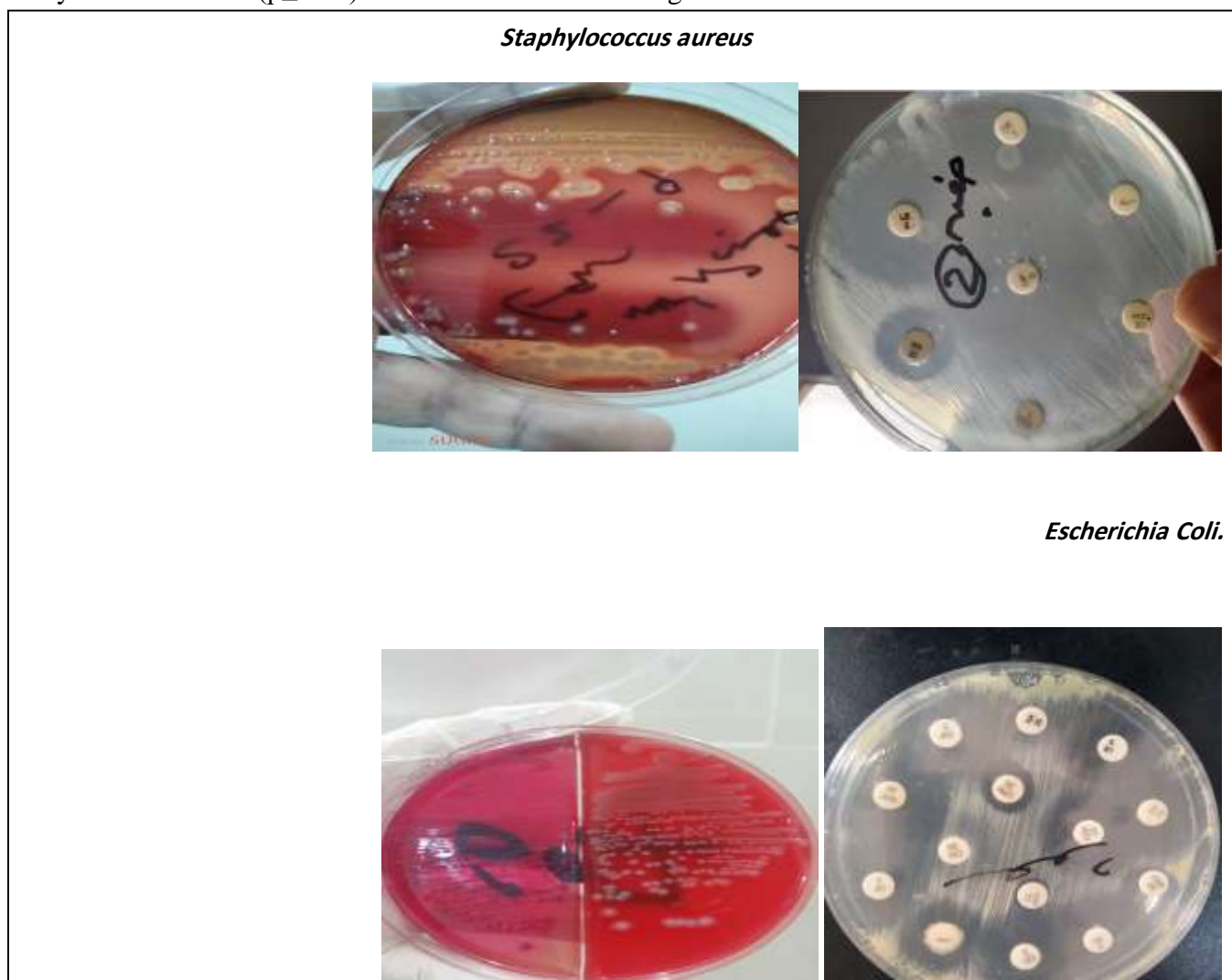


Figure 1: Effect of plant extract on inhibitor of bacteria

The results show that the bacteria *E. coli* was inhibited by the plant extract *Vigna unguiculata*, *Psidium guajava* and the inhibition diameter was 15, and 15.5 mm respectively. (Table2). It was noted that the other plant extracts were of moderate effect against the bacteria *E. coli*.

Table (1) Effect of medical plant on the growth of *staphylococcus aureus*

Plant extract	1mg/L	2mg/L	3mg/L	control
<i>Aloe vera</i>	2	14	18	0
<i>Origanum syriacum</i>	4	16	22	0
<i>Punica granatum</i>	0	0	0	0
<i>Vigna unguiculata</i>	3	12	18	0
<i>Zizphus spina-christi</i>	1	15	20	0
<i>Rumex nervosus</i>	0	9	15	0
<i>Psidium guajava</i>	0	0	0	0

Table (2) Effect of medical plants on growth of *E. coli*

Plant extract	1mg/L	2mg/L	3mg/L	control
<i>Aloe vera</i>	0	6	8	0
<i>Origanum syriacum</i>	0	6	7	0
<i>Punica granatum</i>	0	2	5	0
<i>Vigna unguiculate</i>	2	9	15	0
<i>Zizphus spina-christi</i>	1	6	6	0
<i>Rumex nervosus</i>	1	5	8	0
<i>Psidium guajava</i>	4	10	15.5	0

Effect of plant extract on inhibitor of fungi (*A. niger*):

The results in (Table 3) and figure 2 show the effect of the plant extract used in this experiment against the fungi *A. niger* which causes many health problems. The most effective shown were *Rumex nervosus*, *Origanum syriacum* and *Vigna*

unguiculata, in diameter (22,20,18) respectively in the concentrate 3mg/L. The plant extracts *Aloe vera*, and *Punica granatum* show no effect against this fungi in this study. On the other hand, the plant extract *Zizphus spina-christi* show moderate effect inhibition on the fungi *A. niger* (8mm diameter) in the 3mg/L concentrate.

Table (3) Effect of medical plants on growth of *A. niger*

Plant extract	1mg/L	2mg/L	3mg/L	control
<i>Aloe vera</i>	0	0	0	0
<i>Origanum syriacum</i>	2	15	20	0
<i>Punica granatum</i>	0	0	0	0
<i>Vigna unguiculata</i>	2	10	18	0
<i>Zizphus spina-christi</i>	1	4	8	0
<i>Rumex nervosus</i>	3	14	22	0
<i>Psidium guajava</i>	2	3	8	0

Figure 2: Effect of medical plants on growth of *A. niger*



Discussion

This study indicates that the most plant extract, effective to inhibition this bacteria, was *Origanum syriacum*. This is the first study to investigate the effect of those medical plants (*Origanum syriacum*, *Zizphus spina-christi*, *Aloe vera*, *Psidium guajava* and *Vigna unguiculata*) against the bacteria *Staphylococcus aureus*. In contrast, the previous studies investigated the effect of some medical plants such as the study of **Dalhat et al. 2020** who used the extract of leaves of three important medical plants, *Momordica charantia*, *Ocimum sanctum*, and *Prosopis juliflora*, the studies of **Kamal et al. 2020** which used the extracted leaves of *Ficus carica* and found that this plant is a good source for controlling human pathogenic bacteria. In addition, the study of **Geta and kibret 2020** evaluated the activity of *A. senni* against this bacterium and found that this plant could be a candidate for new antibacterial agents against this bacteria. Moreover, the **Bulugahapitiya et al. 2020** investigated the effect of *D. thawites* against several strains of bacteria and fungi, and found good result as the natural antimicrobial agent. These results agree with **Farhrana et al. 2017** study which found that the effect of the extract *Psidium guajava* was 13.5 mm on bacteria *S.*

aureus and *E. coli* was 12.6 mm in high concentration.

In addition, the results show that the bacteria *E. coli* was inhibited by the plant extract *Vigna unguiculata*, *Psidium guajava*. These results agree with those of **Haq et. al. 2020**, and **Yaseen et al. 2020**, which found that the plant *Aloe vera* had shown significant results of inhibition against bacteria *E. coli*. On the other hand, the extract of *Psidium guajava* show effect against the bacteria strain *E. coli* and the diameter of inhibition was 12.6 mm in the study of **Farhrana et al. 2017**.

Zizphus spina-christi show moderate effect on inhibition of the fungi *A. niger* (8mm diameter) in the 3mg/L concentrate. In respect to the study of **Fabowale et al. 2020**, it was found that the extract of *Trema orientalis linn* is good antimicrobial against bacteria and *A. niger*. The study of **Nasir and Ali 2020** found that the cinnamon extract had high efficacy in inhibition of *A. niger*. The study of **Islam et al. 2015** show the effect of some medical plants against the fungi *A. niger* as well.

Conclusions:

The study is the first study in Yemen to investigate the effect of medical plants such as *Origanum syriacum*, *Zizphus spina-christi*, *Aloe vera*, and *Vigna unguiculata* against the bacteria *Staphylococcus aureus*. The plant extract

Psidium guajava showed good inhibitor on the bacteria *E. coli*. Good results to inhibition of fungi were found. The difference between this study and the previous studies is the method of extraction by using warm water. Some of studies used alcohol for extracting the materials from those plants by using different solvents. The method of extraction could have an effect on effectiveness of those plants against microbes. Further studies should be devoted to investigate the different solvents, effect of the solvents and method of extraction on the concentration of effective materials.

List of abbreviations

Not applicable

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article

Competing interests:

The authors declare that no competing interests exists with respect to this work

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Authors Contributions

ZH prof read the manuscript, HA carried out the laboratory analysis, statistical analysis, writing and edited the work, HA and AA carried out laboratory analysis and prof read the work. M R. carried out plant sampling and carried out chemical and plant sampling. All authors have read and approved the manuscript.

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