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The antibacterial activity of five commercial plant extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract
In the present work, the antibacterial activity of five commercial vegetable extracts (*Allium sativum, Chamaemelum nobile, Thymus vulgaris, Zingiber officinale* and *Ricinus communis*) was evaluated against *Staphylococcus aureus* and *Escherichia coli* by the agar diffusion method, and the microdilution for the determination the minimum inhibitory concentration. For Vincent's method, the diameters of inhibition range between 6 and 20 mm against *Escherichia coli*, in contrary for *Staphylococcus aureus*, the extracts were inactive except garlic extract which is weakly inhibited. The determination of the minimum inhibitory concentration shows that the highest activity was recorded to thyme extract against *Escherichia coli* (108.75 mg / ml), but for *Staphylococcus aureus*, 350 mg / ml of garlic extract was necessary for its inhibition.

Keywords: antibacterial activity, commercial plant extracts, *Staphylococcus aureus*, *Escherichia coli*.

1. Introduction

The use of antibiotics is a major strategy for the eradication of pathogen bacteria and antimicrobial agents are commonly used therapeutically and prophylactically in human medicine therapy (Gyles, 2011) [16]. Today, studies provide in one hand data about antibiotic resistant germs and another hand the resulting risk when this drug was released in the environment (Makky *et al*, 2012; Ghaedi *et al*, 2015) [22, 11]. Drugs have unexpected side effects such as allergy, stomach pain, diarrhea and vomiting (Santos *et al*, 2003). Microbes are all around us and because of their resistance to many antibiotics, it has now become difficult to control them. *Staphylococcus aureus* is a common cause of food poisoning in releasing enterotoxins into food. *Escherichia coli* are bacteria found in the environment, food, and intestines of both humans and animals. *Escherichia coli* cause serious food poisoning in their hosts (Saeb *et al*., 2016) [29]. Bacteria showing resistance to several antibiotics like *Escherichia coli* resistance to third-generation cephalosporins, *Klebsiella pneumoniae* resistance to third-generation cephalosporins and to carbapenems, *Staphylococcus aureus* resistance to beta-lactam antibacterial drugs (WHO, 2014; Amber *et al*., 2017) [35, 2]. The emerging potential of drug resistance of pathogens coupled with high cost and more side effects of antibiotics have drawn the interest of researchers and general population towards ethnomedicinal plants for the potential discovery of useful compounds (Hassan *et al*., 2014) [18]. Traditional medicine rescued and partially replaced the drugs thus reducing their toxic effects. Aromatic and medicinal plants may be an alternative source of antibacterial remedy due to their bioactive compounds (Berroukche *et al*, 2018) [4]. Antibacterial activity of essential oils presents an increasing interest in the last years and was shown to be effective even on multidrug resistant strains (Man *et al*, 2019).

The purpose of the present study is to examine the effect of extracts of *Allium sativum, Chamaemelum nobile, Thymus vulgaris, Zingiber officinale* and *Ricinus communis* on *Staphylococcus aureus* and *Escherichia coli*.

2. Materials and methods

2.1 Commercial extracts

In this study, five plant extracts were used: *Allium sativum, Chamaemelum nobile, Thymus vulgaris, Zingiber officinale* and *Ricinus communis*. These extracts are produced by the Egyptian company El Capitaine (CAPPHARM) for the extraction of oils from natural and cosmetic plants; including the ministerial agreement on health 2848/2002 (https://www.facebook. com/ cappharm/) and Imported by the import-export company Enour of Algeria from Eloued city.

2.2 Test organisms and growth conditions

The five extracts were tested against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300. Bacterial suspensions were made in Mueller Hinton agar (MHA) broth to a concentration of approximately 10^8 CFU/ml using standard routine spectrophometric methods.

2.3 Antimicrobial assays

The disc diffusion method was employed for the determination of antimicrobial activities and microdilution method of the extracts.

a) Screening for antimicrobial activities

Antimicrobial activity was measured with diffusion disk plates on agar, according to Ghannadi *et al*. (2012), with

modifications. In order to test antimicrobial activity, the extracts were dissolved in dimethyl sulfoxide to ½ and ¼. Sterile discs (6 mm in diameter) were impregnated with 7 µL of the extracts (½ and ¼, respectively) with approximately 10^8 bacteria and placed in Petri dishes, over agar and dispersed. Inhibition zones were determined after incubation at 37 °C for 24 h and measured in mm. Negative controls were prepared on discs impregnated with dimethyl sulfoxide (solvent control). Positive control (cultured along with the bacterium without the extract).

b) Minimum Inhibitory Concentration and Minimal Lethal Concentration

The Minimum Inhibitory Concentration (MIC) was performed based on a microdilution method in 96 multi-well microtiter plates according to Chebaibi *et al*. (2016) [7], with modifications. 20 µl of DMSO was added to each of the 96 wells of a microtitre plate, followed by 20 µl of each extract to the first row. This was serially diluted descending down the microtitre plate columns. The initial concentration of tested extract are: [C*T. vulgaris*] = 870 mg/ml, [C *A. sativum*] = 700mg/m, [CC. *nobile*] = 690 mg/ml. The wells of a 96-well which content DMSO and extract were filled with 160 µl of MHB inoculated with 20 µl of exponentially growing culture (about 10^6 colony-forming units/ml).

The wells which content HBM and growing culture serve as positive controls and this which content DMSO and HBM non inoculated were used as negative controls. The microplate was incubated at 37ºC for 18 to 24 h. The MIC was recorded as the lowest concentration of the extract that inhibited the microorganisms' growth after 24 h (Krishnan *et al*., 2010). The minimal bactericidal concentration (MBC) was determined for each of the extracts by sub-culturing the media from each well showing no visible growth onto MHA. The plates were incubated at 37°C until growth was seen in the control plates. The MBC was defined as the consequent concentrations required killing 99.9% of the cells (Scorzoni *et al*., 2007) [32].

3. Results and discussion

The antibacterial activity of commercial extracts from five different plant parts were screened against two bacterial strains, gram positive (*S. aureus*) and gram negative (*E. coli*). The antibacterial potential of extracts was measured in terms of zone of inhibition of bacterial growth. Results obtained with disk diffusion assay regarding the growth inhibition zones of the tested strains are presented in Table1.

Table 1: Antibacterial activities of the extracts against *Escherichia coli* and *Staphylococcus aureus* by disk diffusion method.

Extracts	Pure extract		1/2		1/4		Negative control		Positive control	
	E.coli	***S. aureus***	***E.coli***	***S. aureus***	***E. coli***	***S. aureus***	***E.coli***	***S. aureus***	***E.coli***	***S. aureus***
A. sativum	18	11	10	6	6	6	6	6	6	6
C. nobile	16	6	12	6	6	6	6	6	6	6
T. vulgaris	17	6	12.5	6	9	6	6	6	6	6
Z. officinale	6	6	6	6	6	6	6	6	6	6
R. communis	6	6	6	6	6	6	6	6	6	6

The disc diameter zones of inhibition ranged from 6-18 mm. The maximal inhibition zone was obtained for *E. coli*, however and in except the effect of *A. sativum* pure extract on *S.aureus which* exhibited weak activity no antimicrobial activities of extracts tested against this bacteria has been revealed. *A. sativum, T. vulgaris* and *C. nobile* pure extracts have the largest zones on *E. coli* with zones of 18, 17 and 16 mm, respectively. *Z. officinale* extract 6 mm and *R. communis* extract 6 mm and did not show inhibition against *E. coli* and *S. aureus*. For ½ dilution of extracts, *A. sativum, T. vulgaris* and *C. nobile* exhibited an inhibition on *E. coli* with a diameter ranged between 10 and 12.5 mm.

These essential oils showed antibacterial activity against the tested strains with an MIC and MBC in the ranges of 108.75-175 mg/ml and 435–700 mg/ml, respectively for *E. coli*. For *S.aureus*, only *A. sativum* extract was active on tis bacteria with an MIC and MBC of 350 mg/ml. The most active extract was *T. vulgaris* oil with a MIC and MBC of 108.75 mg/ml and 435 mg/ml, respectively for *E. coli*. *A. sativum* extract were less effective against *E. coli* and presented a MIC of 175 mg/ml and a MBC 700 mg/ml.

Table 2: Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *A. sativum, T. vulgaris* and *C. nobile* extracts.

Extracts	MIC		MBC	
	S. aureus	***E.coli***	***S. aureus***	***E. coli***
A. sativum	350 mg/ml	175 mg/ml	350 mg/ml	700 mg/ml
T. vulgaris	/	108.75 mg/ml	/	435 mg/ml
C. nobile	/	172.5 mg/ml	/	690 mg/ml

The present study revealed the antibacterial activity of *A. sativum* extract against *E. coli* and *S. aureus*. Disc diffusion and microdilution assay demonstrated that the *A. sativum* extract was more effective than the other extracts. There are Similarities and differences between these results and the resembling studies. Ankri *et al*. (1999) tested aqueous and ethanolic extracts of *A. sativum* against *E. coli* and *S. typhi*, and found that the aqueous extract had little or no inhibition while the ethanolic extract had a higher antibacterial activity. Nedorostova *et al*. (2009) [26] in their study of the activity of the essential oils of 27 plants species against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, and *S. enterica Enteritidis* ATCC 13076, found that *A. sativum* essential oil was active, on all tested bacteria. Jagadeesh babu*et*

al. (2011) reported that clove, cinnamon and garlic had the antibacterial activity against various bacterial pathogens *Staphylococcus aureus*, *E.coli*, *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter jejuni*. Karuppiah and Rajaram. (2012) [20] investigated the antibacterial efficiency of *A. sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. The results showed that *A. sativum* extract inhibit *E.coli* and *S.aureus* with highest diameter of zone of inhibition of 18.50 mm 14.55 mm respectively.

Many researchers in different countries report that *A. sativum* extract was active on *S. aureus* and *E. coli* (Sah *et al*. (2012) [30] from Sultanate Omane, Gull *et al*. (2012) [15] from Pakistan, Garba *et al*. (2013) from Sokoto, Nigeria, …). However, the antimicrobial activity of garlic has been attributed to the presence of thiosulfinates (e.g., allicin) whose removal completely renders garlic ineffective against microorganisms (Alli *et al*., 2011; Khadri *et al*., 2010) [1, 19].

In our study, *E. coli* was more sensitive than *S. aureus.* In general, Gram-positive bacteria were found to be more sensitive to EOs or antibacterial compounds than Gram-negative bacteria because of the differences in cell structure, which may retain the entry of hydrophobic compounds in the cell (Burt, 2004, Cox and Markham, 2007, Dorman and Deans, 2000) [6, 8, 9]. Many studies report that *A. sativum* essential oil act more on Gram-positive than Gram-negative bacteria. In a study shown that there is no general rule with respect to the Gram sensitivity because many controversies exist in the different published works (Najafi *et al*., 2016) [24]. Our results regarding the antimicrobial activity of *T. vulgaris* extracts on *E. coli* and *S. aureus* are in agreement with those previously reported by Nascimento *et al*. (2000) [25] and Touri *et al*. (2018) [33], who their studies demonstrated that thyme essential oil did not have any inhibitory effect on *S. aureus*. The antimicrobial activity of *T. vulgaris* essential oils could be related to the thymol content, which has been tested previously and was found to have a significant antibiotic activity (Rota *et al*. 2011; Guarda *et al*. 2011; El-Jalel *et al*. 2018) [28, 14, 10]. Also, the synergistic effect between the different oil's compounds, i.e., thymol and carvacrol (Guarda *et al*. 2011) [14] and p-cymene and carvacrol (Ultee *et al*. 2002) [32] has also been reported. Several studies demonstrate the antimicrobial effects of *C. nobile* extract against different bacterial strains. The antimicrobial activity of essential oils of the flower of *C. nobile* from the Provence (France) was tested by Bail *et al*.(2009) [5] against various strains of Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella* sp.) by a modified agar dilution and agar diffusion method. Their results showed high antimicrobial activity against all strains of tested microbes. In another study, the volatile oil of *C. nobile* showed an activity (filter paper diffusion test) against Gram-positive bacteria, especially *Bacillus subtilis*, *B. anthracis*, *Micrococcus glutamicus*, *B. sacchrolyticus*, *B. thuringiensis*, *Sarcina lutea*, *B. stearothermophilus*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Staphylococcus* sp. and *L. casei*, whereas the oil showed no activity against Gram-negative bacteria species including *Salmonella* group B, *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Pseudomonas* sp., *Salmonella saintpaul* and *Salmonella weltevreden* (Hänsel *et al*., 1993) [17].

The antimicrobial activity of *C. nobile* extracts could be due to the presence of different phenolic compounds, coumarins, flavanoids and their derivatives (Rizwana *et al*., 2016) [27].

4. Conclusion

The commercial extracts from *Allium sativum, Chamaemelum nobile, Thymus vulgaris* exhibit promising antimicrobial effects against selected bacteria (*Staphylococcus aureus* and *Escherichia coli).* The *Allium sativum* extracts particularly may be considered as possible sources for the development of new antimicrobial agents for the treatment of several infectious diseases and contaminations caused by those pathogens germs.

5. References

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