

# Effect of phenolic extracts of Algerian medicinal plants on the bacterial growth and adherence of *Staphylococcus aureus* and *Bacillus cereus* pathogens responsible of food poisoning.

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## Abstract

Artemisia, one of the larger genera in the family Asteraceae, comprises from 200 to more than 500 taxa at the specific or subspecific level. *Artemisia herba-alba* Asso (Shih) is grown in North Africa and certain parts of Asia and Middle East. It is one of the most widely used plants in the Algerian folk medicine. The antioxidant and free radical scavenging activities of the methanolic extracted materials were tested together with their antibacterial effects against isolated bacterial strains causing food poisoning. In summary, *A. herba-alba* Asso represent a good phenolic and flavonoid content (161, 64 mg/g and 16,83mg/g, respectively) in comparison with *T. capitatus* L which represent a phenolic content of 131, 48 mg/g and 14, 96 of flavonoid content. The results shows also that the methanolic extracts of the two plants possess a strong antioxidant (DPPH, FRAP assays) and antibacterial activities, which supports their ethnopharmacological use and *A. herba alba* represent the highest values (IC 50: 2, 35 mg/ml and OD 1, 13 at 700nm). Our results show the interest of *A. herba-alba* Asso and *T. capitatus* L, among other medicinal plants, in search of new chemo-preventive agents against biofilm and planktonic growth of food spoilage pathogens. Further studies are envisaged to target the most interesting molecules responsible for these activities. It is concluded that organic extracts from *Artemisia herba alba* Asso, exert strong antioxidant activities which are related to their polyphenol contents. These findings demonstrate the remarkable potential of these plants as valuable source of Natural antioxidants.

**Keywords:** food poisoning, *S. aureus*, *B. cereus*, *A. herba alba*, *T. capitatus*, Antioxydant.

## Introduction

Foodborne illness is a major problem with enormous associated costs. Foodborne pathogens occur widely in nature and it is difficult to prevent them from entering raw foods. *Staphylococcus aureus* is a Gram-positive bacterium and is considered one of the most prevalent causes of gastroenteritis worldwide [1], with milk and dairy products and meat products (particularly cooked and fermented) being some of the most frequently involved foods. *Bacillus cereus* belongs to a genus of Gram-positive, endospore-forming bacteria, which is involved in foodborne outbreaks with variable incidence from country to country [2]. Medicinal plants primary and secondary metabolite constituents have a long history of use in old and modern medicines and in certain systems of traditional medicines, and are considered the sources of important medications such as vincristine, codeine, quinine, morphine, digoxin, atropine and others<sup>1</sup>. In Algeria *Artemisia herba-alba*, and

thyme (*T. capitatus*) are largely used in traditional treatment of digestive diseases and culinary but not very well studied scientifically. *Artemisia herba-alba* and thyme (*T. capitatus*) are aromatic plants naturally grown in mountain areas of Algeria. *A. herba-alba* is used for treatment of gastric disturbances, such as diarrhea, abdominal pain and for healing external wounds. It is also used as remedy for gastritis and inflammation of the gastrointestinal tract [3]. The thyme has traditionally been considered as an anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, rubefacient, sedative, stimulant, and tonic. The plant has been used as a folk medicine against asthma, arteriosclerosis, colic, bronchitis, coughs and diarrhea [4]. On the other hand, phenolic compounds, in addition to their antimicrobial properties, are known to have antioxidant ability because of their role as free radical scavengers. Their antioxidant potential depends on the number and arrangement of the hydroxyl groups and the extent of structure conjugation [5]. A reagent with a triple function as flavoring, antioxidant (preventing the chemical spoilage of food) and antimicrobial (against pathogenic and spoilage

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microorganisms) would be of interest to the food industry, because it would enable the total amount of additives used in foods to be reduced.

## Materials and Methods

### Isolation of *Bacillus cereus* strains from different Food Sources

Several Samples from local commercial supermarket and home-made foods were collected. The used foods are mainly: fresh and raw ground meats, poultry, fish, dairy products and some cooked dishes. All food samples were transported in sterile plastic boxes. The isolation of *Bacillus* species was performed according to the conventional procedure by serial dilution in sterile phosphate buffered saline. 10 g of food sample was added in 90 mL of phosphate buffered saline; the different solutions were heated at 85 C for 10 min. 100µl from the appropriate dilutions were surface plated on LB agar, and on Mannitol egg-yolk polymyxin B (MYP) agar plates [6]. The isolation of *S. aureus* was done by the method described previously using Baird parker agar, after a 10-fold serial dilution, a volume from each dilution was spread onto plate containing Baird Parker (BP) agar with 20% egg yolk telluride emulsion by drop plating technique. Plates were incubated aerobically at 37 C for 24 - 48 h. [7,8]. The characterization of the isolates was performed by studying colonies morphology, Gram stain, lecithinase, hemolysis on blood agar, coagulase production and biochemical tests, using API 20 E, API Staph system and Bergey's Manual of Systematic Bacteriology. [9].

### Screening of *Bacillus cereus* and *Staphylococcus aureus* virulence

The hemolytic activity was assessed on blood agar medium (The isolates were spotted on blooded nutritive agar medium. [10]. After incubation at 33 C for 18 h. The strains were classified as (partial), β (total), or non-hemolytic. The production of phospholipase-C was measured by adding egg yolk emulsion up to 5% (v/v) to nutritive agar. Each isolate was spotted on the medium and the plates were incubated at 30 C. Opaque zones around the colonies, caused by hydrolysis of lecithin indicated lecithinase production. [11]. Caseinase activity was identified according to the method of Gudmundsdóttir [12] on milk agar medium. The isolated bacteria were streaked on the appropriate medium for 24 h at 37 C. A transparent zone around the colonies indicated caseinase activity. The ability to hydrolyse starch was tested by inoculating on the starch agar medium; the zones were detected by adding lugol to the plate's surfaces. [10].

### Coagulase test tube

Confirmation of the genus, *Staphylococcus* was done by Gram staining and various biochemical tests, while the species, *S. aureus* was confirmed by Coagulase test as described by Monica [13].

### Original CRA (CRAori) test

*S. aureus* strains were cultivated on BHIA (Difco) with 0.08 % (w/v) Congo red (Sigma- Aldrich, Germany) supplemented with 5% (w/v) sucrose. The strains were inoculated in streaks and incubated at 35 C under aerobic conditions for 24 and 48 h. The staphylococci bio film producer strains formed black colonies, while the non-bio film producer strains formed red colonies. The stains were incubated at 35 C under microaerophilic conditions and were evaluated after 24 and 48 h [14].

### Plant material

The plant materials were studied following its large use in Algerian culinary and its potential medicinal uses. The plant used in this study consisted by *Thymus capitatus*, collected during spring season from the region of Safsaf (Mostaganem, Algeria), *Artemisia herba alba* (Asso) from the region of Saida, Algeria. The identification of the different plant studied was done at Microbiology and vegetal biology Laboratory at Mostaganem University. The plants were washed with distilled water and dried at room temperature under shade. The dried aerial parts were powdered by a blender and stored away from light for further studies.

### Extraction procedure

70% methanol (v/v), was used to extract phenolic compounds. 10% w/v of powdered plant material was extracted by 100 ml of solvent. The extraction of both plants *A. herba alba* and *T. capitatus* was done under reflux using methanol as solvent. The extraction was performed during 30 min. Centrifugation was done at 3000 rpm/20min, and vacuum filtration was used to separate the liquid extract. The filtrates were collected and the solvent was evaporated at 40 C using a rotary vacuum evaporator.

### Total phenolic and Flavonoid content

Phenolic content of the obtained extracts was determined by the method of Singleton and Rossi, [15]. Gallic acid concentrations were prepared as standard in methanol. To determine the flavonoid content (TFC), the aluminum chloride colorimetric assay was used as described by Liu et al. [16]. Quercetin was used for the calibration curve. The results of flavonoids content were expressed as mg quercetin equivalents (QE) per g. All the samples were analyzed in triplicate.

### Measurement of radical-scavenging activities



The antioxidant activity of the phenolic compounds used as antimicrobials was determined according to two procedures: 1) the scavenging of DPPH radical, 2) the FRAP method. The scavenging of DPPH radical was carried out as described by Shimada et al., [17]. The FRAP method (procedure 2) was conducted according to the procedure of Oyaizu [18]. All data were expressed from three assay methods.

### Antimicrobial activity of plant extracts

Agar-well diffusion assay: The antibacterial activity of the different plant extracts was evaluated against five isolates of food spoilage *Bacillus cereus* and five of *Staphylococcus aureus*. The turbidity of the bacterial suspensions was adjusted to an equivalent to 0.5 McFarland. The agar-well diffusion assay was performed according to the recommended method of Valgas et al. [19]. A standardized bacterial inoculum was uniformly surface spread on a Mueller-Hinton agar. Next, 80 µL of each plant extract dissolved in distilled water (10mg /ml) was added into the wells of 6 mm in diameter, the plates were incubated at 30 C for 24h and the measure of the diameter of inhibition zones was done. Well containing the solvent and water only were used as controls. All tests were done in triplicate.

### Effect of plant extracts on the adherence and planktonic growth of *B. cereus* and *S. aureus*

This test was used to detect the ability of bacteria to adhere to smooth surface (glass tubes), and to evaluate the effect of *A. herba alba* and *T. capitatus* extracts on the adherence and planktonic growth of the tested strains. The test was done by cultivation the tested bacteria in glass tubes containing (2ml) of Trypticase soy broth (TSB) with 200µl of the different concentrations of plant extracts (10µL-200µL) obtained after dilution of a solution stock (2mg/ml) of each plant extract. The control tube contains (2ml) of TSB without extracts. All the tubes were incubated for 24 h at 37°C. After incubation, the absorbance of the bacterial suspensions was read at 600 nm. After that, the contents of tubes were removed and tubes were stained by adding (2 ml) of 0.1% crystal violet for 30 minutes, then the effect of plant extracts on adhering bacteria was seen. Slime production was visible through a

film that occurred on tube walls. After 30 minutes, the dye was removed and the wells were washed thoroughly, 2 ml of 95% ethanol was added and incubated for 15 minutes. The reaction mixture was read at 595 nm. [20].

### Statistical analysis

All the experiments were carried out in triplicate. The data were analysed by ANOVA. The IC50 values were calculated from linear regression analysis. Correlations between variables were established by excel.

## Results

### Isolation and identification of the isolated *Bacillus cereus* and *S. aureus* strains

24 isolates of *B. cereus* and 18 of *S. aureus* were selected after screening of their ability of resistance to penicillin G and other antibiotics (data not shown). 5 isolates of each genus showing high resistance to the different antibiotics were selected for this study. The colonies of isolated *B. cereus* bacteria were big waxy white or gray, surrounded by an opacity zone. Unable to catabolize mannitol when cultured on MYP agar. The isolates were gram positive rods. Motile, and grow positively in 7% NaCl but could not grow at 50 C. (Table 1). While, the colonies of *S. aureus* isolates were black with an opacity zone, all *Staphylococcus aureus* isolates were found able to ferment mannitol sugar and positive to coagulase, and 12 strains (66,66%) of *S. aureus* were able to form biofilm when were cultivated on CRA agar. The isolates were gram positive cocci not motile and were able to grow in 7, 5 % NaCl (Chapman agar). The presence of *S. aureus* in these samples was determined by plating on BP agar, followed by confirmation via biochemical tests. (Table 2). The identification of the above species was confirmed by API 20 E and API Staph system as recommended by bergy's manual. All *Bacillus cereus* and *S. aureus* stains were beta-hemolytic producing phospholipase C, amylase and caseinase. The obtained results indicate that the studied *Bacillus cereus* and *S. aureus* strains are carrying a level of toxicity and virulence.



**Table 1:** Identification of isolates of food spoilage *Bacillus cereus*

	Gram-endospore	Catalase	Oxydase	Mobilité	50°C	Indole	VP	Citrate	Urease	Nitrate	OF	7%NaCl	Man
BCSV1	+	+	+	+	-	-	+	+	-	+	+	+	-
BM18	+	+	+	+	-	-	+	+	-	+	-	+	+
BF15	+	+	+	+	-	-	+	+	-	+	+	+	-
BL5	+	+	+	+	-	-	+	+	-	+	+	+	-
BV12	+	+	+	+	-	-	+	+	-	+	+	+	-
LC2	+	+	+	+	-	-	+	+	-	+	-	+	+
BVDE17	+	+	+	+	-	-	+	+	-	+	+	+	-
BRIS7	+	+	+	+	-	-	+	+	-	+	+	+	-
BY5	+	+	+	+	-	-	+	+	-	+	+	+	-
BLB1	+	+	+	+	-	-	+	+	-	+	+	+	-
BF12	+	+	+	+	-	-	+	+	-	+	+	+	-
BS2	+	+	+	+	-	-	+	+	-	+	-	+	+
BP14	+	+	+	+	-	-	+	+	-	+	+	+	-
BV22	+	+	+	+	-	-	+	+	-	+	+	+	-
BLP2	+	+	+	+	-	-	+	+	-	+	+	+	-
BM23	+	+	+	+	-	-	+	+	-	+	+	+	-
BC13	+	+	+	+	-	-	+	+	-	+	-	+	+
BSM11	+	+	+	+	-	-	+	+	-	+	-	+	+
BL3	+	+	+	+	-	-	+	+	-	+	+	+	-
BFMG16	+	+	+	+	-	-	+	+	-	+	+	+	-
BSEM4	+	+	+	+	-	-	+	+	-	+	+	+	-
BRIS3	+	+	+	+	-	-	+	+	-	+	+	+	-
BM11	+	+	+	+	-	-	+	+	-	+	+	+	-
BM2	+	+	+	+	-	-	+	+	-	+	+	+	-

**Table 2:** Identification of isolates of food spoilage *Staphylococcus aureus*

	Gram	Catalase	Oxydase	Mobilité	Indole	V P	Nitrate	7,5%NaCl	Man	Hémolyse	Coagulase	Urease
SVDE10	+	+	-	+	-	+	+	+	+	+	-	+
SYDN1	+	+	-	+	-	+	+	+	+	+	+	+
SPTS2R	+	+	-	+	-	+	+	+	+	+	+	+
SVLY7	+	+	-	+	-	+	+	+	+	+	+	+
SEPC7	+	+	-	+	-	+	+	+	+	+	-	+
SLV5BP	+	+	-	+	-	+	+	+	+	+	+	+
SVDE7	+	+	-	+	-	+	+	+	+	+	+	+
SLVC15	+	+	-	+	-	+	+	+	+	+	+	+
SFMG2	+	+	-	+	-	+	+	+	+	+	-	+
SLGM1 1	+	+	-	+	-	+	+	+	+	+	+	+
SSEM2	+	+	-	+	-	+	+	+	+	+	+	+
SRSC5B	+	+	-	+	-	+	+	+	+	+	-	+
SLVC9	+	+	-	+	-	+	+	+	+	+	-	+
SVDE9	+	+	-	+	-	+	+	+	+	+	+	+
SEPC4	+	+	-	+	-	+	+	+	+	+	+	+
SCHR7	+	+	-	+	-	+	+	+	+	+	+	+
SFMG1 4	+	+	-	+	-	+	+	+	+	+	+	+
SYLG12	+	+	-	+	-	+	+	+	+	+	+	+

## Total phenolic and Flavonoid content

The results in figure (1) show the extraction yield of total phenols and flavonoid from the different plant extracts. This yield increase

with the increase of the concentration of the extracts used in this study and *A. herba alba* represents the highest content of polyphenols and flavonoids (161, 64 mg/g and 16,83mg /g) followed by *T. capitatus* (131, 48 mg/g and 14,96).



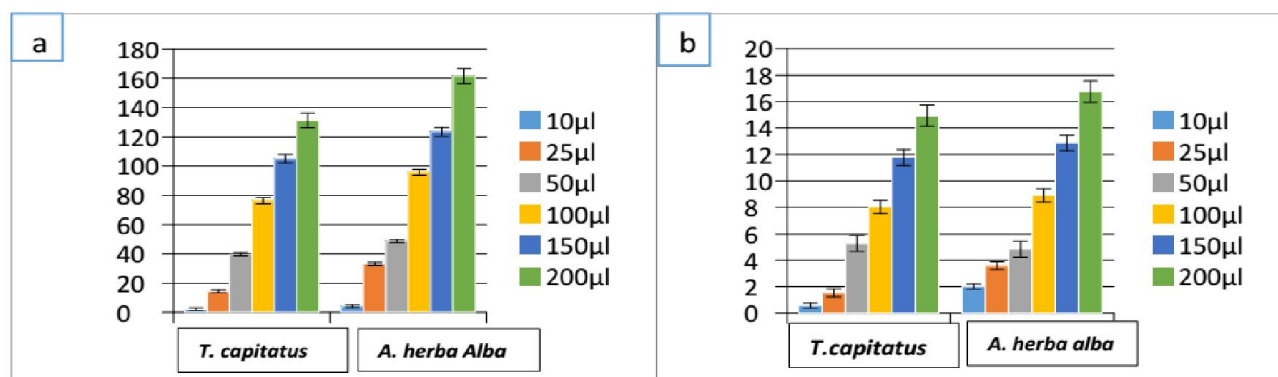


Figure 1. Total phenolic content (a) and flavonoid content (b) of *T. capitatus* and *A. herba alba* extracts.

### Measurement of radical-scavenging activities

To evaluate the antioxidant activity, two methods were used DPPH and FRAP. For the first method and in comparison with ascorbic acid (AA) as positive control, it was found that methanolic extract of *A. herba alba* represented the highest DPPH scavenging activity (91, 65%  $\pm$  0,001), this value was significantly different from *T. capitatus* extracts which represent 80,13%  $\pm$  0,027. (Figure. 2). These values were lower than those obtained by ascorbic acid (positive control) which represent 91, 79%. IC 50 values obtained

for the investigated plant extracts, methanolic extract of *A. herba alba* was found to be 2, 35 mg/ml while *T. capitatus* extracts was 13,62 mg/ml and ascorbic acid represent a value of 12,21 mg/ml. The results indicate that the scavenging ability of methanolic extract of *A. herba alba* (Asso) on DPPH radical was strong. The results of FRAP method confirmed those obtained by DPPH assay and the extract of *A. herba alba* (Asso) represented the highest values of DO (0, 16-1, 13) at 700 nm (Figure 3). These results were correlated with the results of phenolic contents.

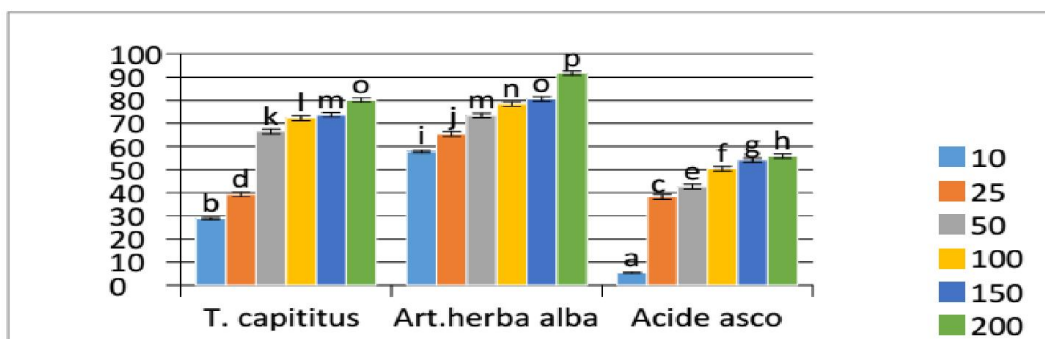


Figure 2. Scavenging ability of different plant extracts.

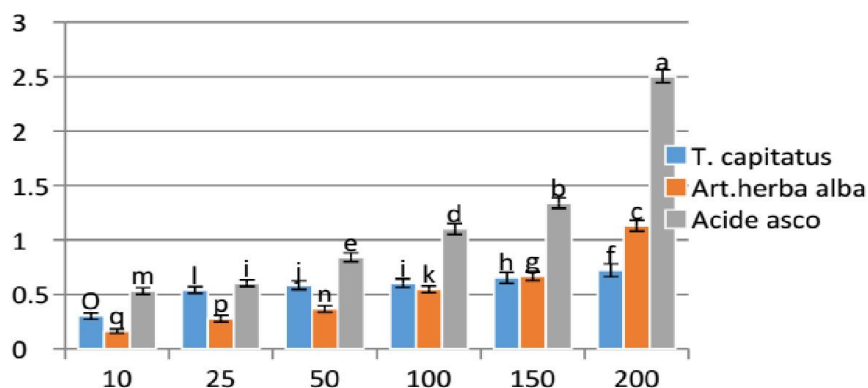


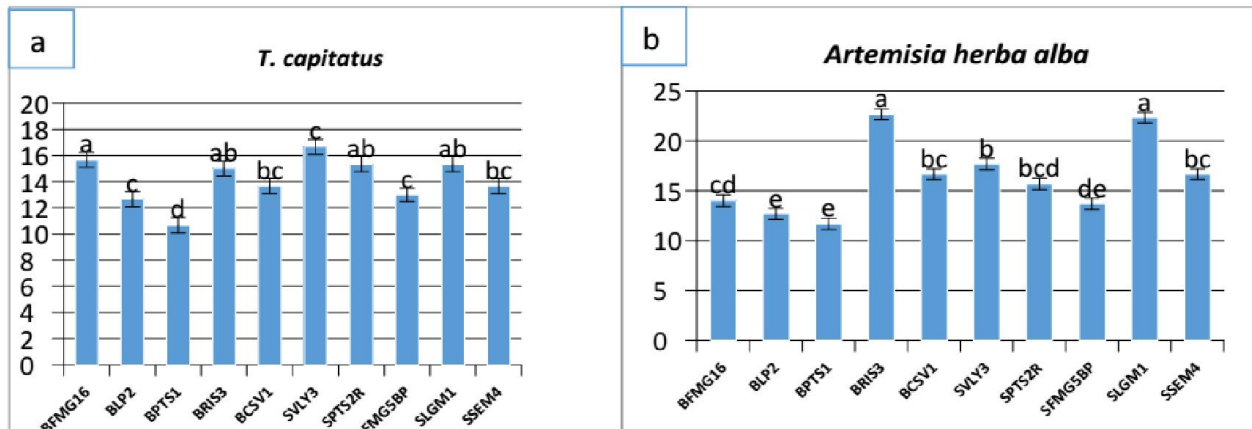
Figure 3. Reducing power of plant extracts.



## Antimicrobial activity of plant extracts (Agar-well diffusion assay)

The results of antibacterial activity against strains of *Bacillus cereus* and *S. aureus* are illustrated in figure 4. The inhibition zones of

bacterial strains obtained by the *A. herba alba* extract were in the range of  $10,33 \pm 0,05$  to  $12,66 \pm 0,05 \pm 1$  mm, which was significantly different from those obtained by a *T. capitatus* extracts with  $7 \pm 0,05$  to  $9 \pm 0,05$ , respectively. It can be observed that the extracts studied possessed an inhibitory effect on all tested strains, depending on plant extracts and amounts of phenolic compounds.



**Figure 4.** Antimicrobial activity of *T. capitatus* (a) and *Artemisia herba alba* (b) extracts against isolates of *Bacillus cereus* and *Staphylococcus aureus*.

## Effect of plant extracts on the adherence and planktonic growth of *B. cereus* and *S. aureus*

According to the obtained results we found that both methanolic extracts *Thymus capitatus* and *Artemisia herba-alba* inhibited bacterial growth of different isolates of the two species studied. The results presented in the figure (5 and 6) showed that in the case of low concentrations (0.5 mg / ml) of the tested extracts, a small decrease in bacterial growth of *Bacillus cereus* and *Staphylococcus aureus* strains (OD 1.12 to 1.09) and (OD of 1.54 to 0.75), respectively, was observed by the *Thymus capitatus* extract. About *Artemisia herba-alba* the OD values obtained are 1.21 to 1.12 for *Bacillus cereus*, and 1.09 to 0.75 for *Staphylococcus aureus*. A significant reduction in bacterial biomass formed by the two strains studied was observed in the presence of methanol extracts of *Thymus capitatus* and *Artemisia herba alba* at a concentration of 2 mg/ ml. From the results obtained in the inhibition of bacterial growth of *Staphylococcus aureus* and *B. cereus* by the two plant extracts, we found that these inhibition differ from strain to another. The results presented in the figures 7 and 8 have shown that biofilm formation decreases with the increase of the concentration of methanolic extracts of *Thymus capitatus* and *Artemisia herba-alba*, except for BFMG16, BPTS1, SVLY3 and SSEM4 strains that the bacterial biofilm grew in the presence of plant extracts studied, which explains that these strains are resistant to our extracts tested. The antibiofilm activity of *Artemisia herba-alba* extract was

higher on both bacterial species than the extract of *Thymus capitatus*. We tested by the method of crystal violet the capacity of ten (10) different strains of our cohort belonging to *Bacillus cereus* and *Staphylococcus aureus* to form in vitro bacterial bio films in the presence of extracts from *Thymus capitatus* and *Artemisia herba-alba*. For inhibiting biofilm formation most of the tested bacterial strains was lower in the case of the concentration (0.5 mg / ml) and for *Bacillus cereus* strains, the percentage inhibition obtained for the extract *Thymus capitatus* was 7.19 up to 22.48% for the *Artemisia herba-alba* was 23.41 to 40.10%. The percentage of biofilm formation inhibition obtained with the extract of the plant *Thymus capitatus* against the growth of *Staphylococcus aureus* is 10, 20% to 56.75% for the extract of *Artemisia herba-alba* the values obtained are 1.13% up to 34.59%. At high concentrations (2 mg/ ml), the percent of inhibition of biofilm formation was raised to extract *Thymus capitatus* was 26, 02% up to 50.33%, while the *Artemisia herba-alba* represented a percentage of inhibition of 38.82% to 59.42% vis-a- vis the various *Bacillus cereus* isolates. On *Staphylococcus aureus* strains were less sensitive to different concentrations of both methanolic extracts tested. The percentage inhibition obtained with the extract of *Thymus capitatus* was 21.12% to 67.07%, and 13.59% up to 47.53% were obtained by the extract of *Artemisia herba-alba*. All these results showed that the percentage of inhibition of biofilm formation by both extracts of the test plants, increases with increasing the concentration of the methanol extract of each plant extract of *Artemisia herba-alba* was more effective at inhibiting biofilm formation than *Thymus capitatus*.

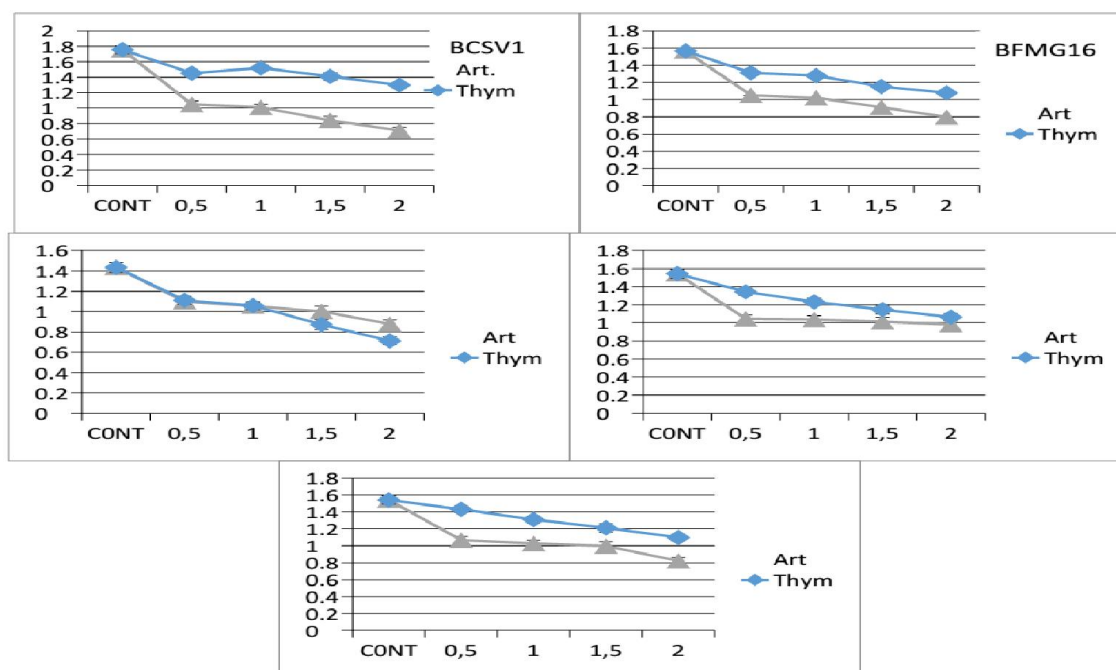


Figure 5. Inhibition of planktonic growth of *Bacillus cereus* by methanolic extract of *Artemisia herba-alba* and *Thymus capitatus*.

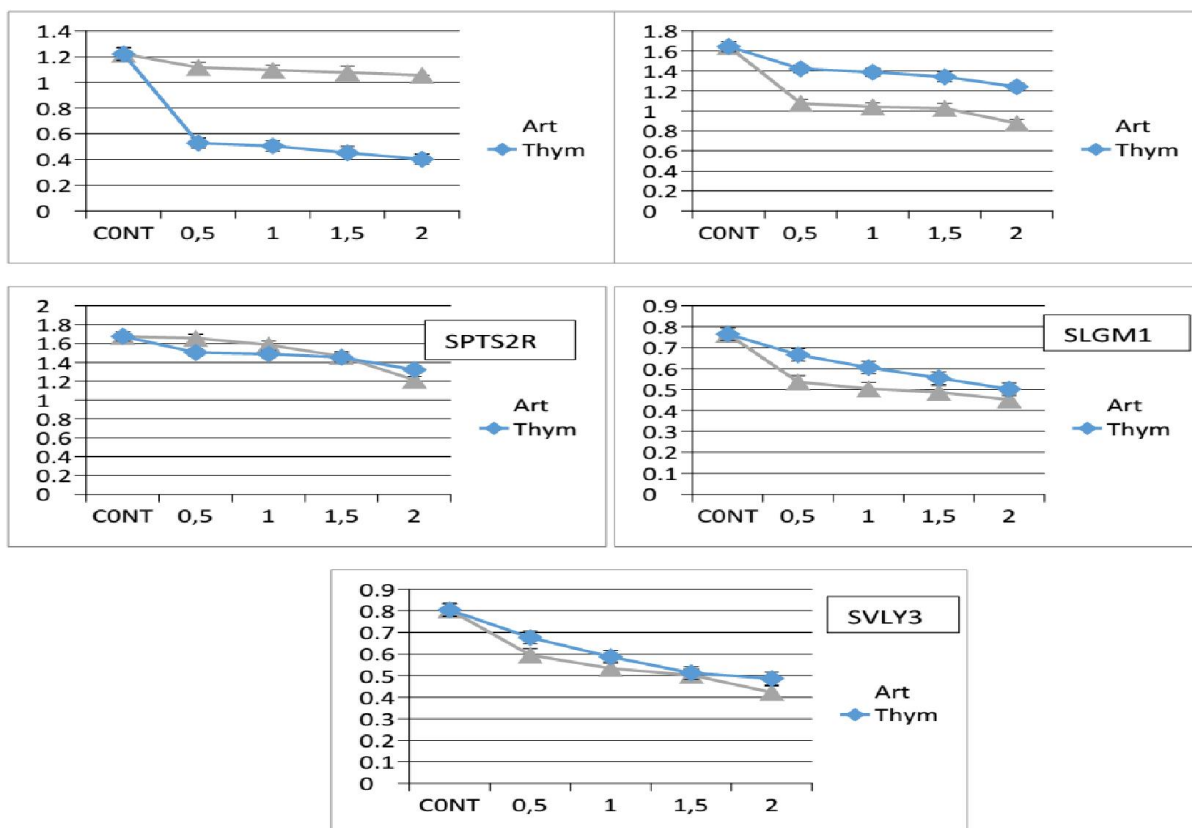


Figure 6. Inhibition of planktonic growth of *Staphylococcus aureus* by methanolic extract of *Artemisia herba alba* and *Thymus capitatus*.

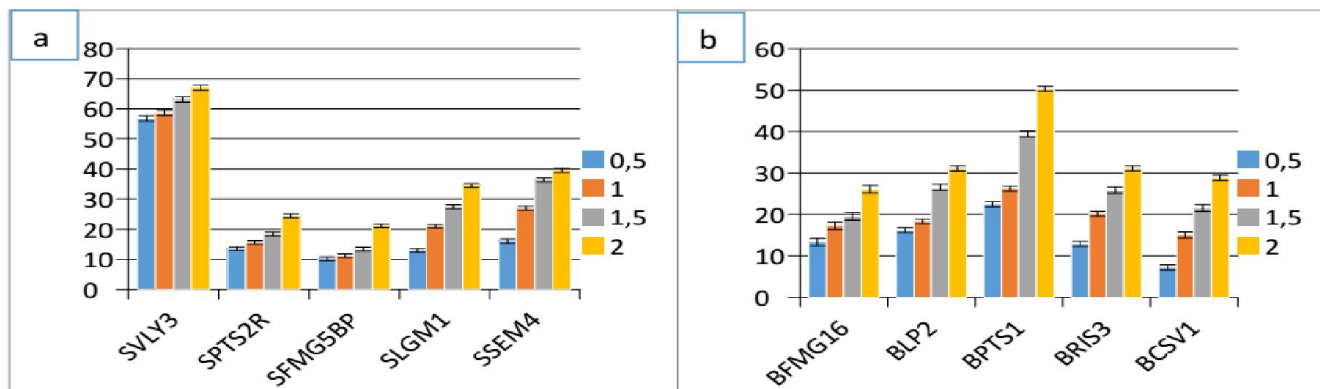


Figure 7. % Inhibition of mature bio films of *Staphylococcus aureus*(a) and *Bacillus cereus*(b) by the methanolic extract of *Thymus capitatus*.

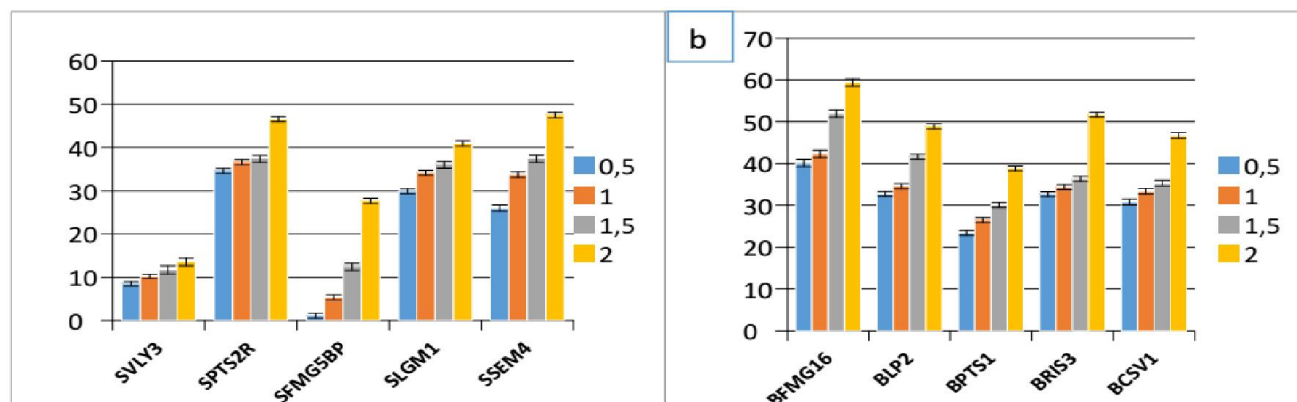


Figure 8. % Inhibition of mature bio films of *Staphylococcus aureus*(a) and *Bacillus cereus*(b) by the methanolic extract of *Artemisia herba-alba*.

## Discussion

Spices are important vectors for various microorganisms implicating possible health problems for consumers as well as quality and shelf-life problems for foods. Studies on the microbiological quality of spices demonstrated profiles of microorganisms, including total heterotrophs, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Shigella* [21, 22]. Meat and milk products are found to be among the most frequently involved matrices during outbreaks of food poisoning [23, 24]. 13 staph enterotoxins foods that have been frequently incriminated in staphylococcal intoxication include meat and meat products, poultry and egg products, milk and dairy products, bakery products, particularly cream filled pastries and cakes salads, , and sandwich fillings [25, 26]. *B. cereus* is an important underestimated foodborne pathogen that is ubiquitous in the environment [27]. The contamination of milk and other dairy products with *B. cereus* is a common problem due to its effect on the quality of the products and the potential health hazards of the presence of toxigenic strains [28]. The pathogenicity of foodborne *S. aureus* is associated with the ability of some strains to produce enterotoxins [23]. The resistance to antimicrobials, particularly to  $\beta$ -lactam antibiotics, has also raised high concern as

an emerging problem in the food environment [29]. According to the results of the present study, most *B. cereus* group strains produced extracellular enzymes, such as protease, lipase, lecithinase, gelatinase and amylase. In Italy, Cosentino et al. [30] reported that nearly all strains with their origin in dairy products origin showed a strong enzymatic activity by hydrolyzing casein, gelatin, starch, and olive oil. In addition, Molva et al. [31] noted that most of *B. cereus* and *B. thuringiensis* strains from cheese samples were found to produce lecithinase, gelatinase, lipase and protease. In another survey, De Jonghe et al. [32] investigated the production of the spoilage enzymes that adversely affect milk quality among the *Bacillus* species. *Bacillus* strains were strongly proteolytic and lipolytic, but lecithinase activity seems to be the less abundant trait. Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood [33]. Following Ross et al. [35], a great number of phenolic compounds can be obtained by methanol extraction such as phenolic acids, flavanons, flavanols, anthocyanins, catechins, and procyanidins. The quantification of the total phenolic content in the extracts showed that the aqueous mixtures at 70% solvent and in comparison with pure solvents, were more effective to extract the phenolic compounds. [36]. In fact, recent studies showed that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity



of many medicinal plants [37]. Thus, our data support conclusions of others who attributed antioxidant activities to the presence of phenolic compounds in thyme [38, 39]. Recent studies showed that natural antioxidants such as polyphenols are often added to foods to stabilize them and prevent off-flavour development and considerable interest for their potential role as functional foods or nutraceuticals [40]. In fact, it has been found that antioxidant molecules such as polyphenols, flavonoids, and tannins reduce and discolour DPPH due to their hydrogen donating ability [41]. These results may be due to hydroxyl groups existing in the chemical structure of phenolic compounds from *T. capitatus* extracts that can provide the necessary component as a radical scavenger [42, 43, 44]. Thymol is a natural phenolic monoterpene, known for its antioxidant [45] and anti-inflammatory [46] properties. It also has a significant effect against several pathogens such as bacteria [47]. Phenolic compounds were reported to be very strong antioxidants [48]. The correlation between antioxidant activity and total phenol contents has been largely studied in different food products. [49]. It has been reported that amount of total phenolic compounds in Asteraceae varieties is higher than in other families. Indeed, amounts of total phenolic compounds in extracts of plants we tested were higher compared to what was reported in other plants [34]. Interestingly, the amounts of total phenolics and flavonoids in *A. herba-alba* extract were higher compared to what was found elsewhere [50]. Al Mustafa and Al Thunibat [51] found a phenolic amount in methanolic extracts from *A. herba-alba* shoots fourfold lower than what we measured. These differences may be due to variability of phenolic metabolism in different *A. herba-alba* organs, but also to differences in climate conditions (hot temperature, high solar exposure, dryness, short growing season) between Central Tunisia and other countries. It was reported that *Artemisia herba-alba* has a weaker antibacterial activity than the related species of *Artemisia* [52, 53]. Antibacterial activities of flavonoids were described previously [54]. Various plant products, particularly spices and extracts of various herbal plants were widely utilized as natural antimicrobials and antioxidants [55]. The demand of these herbal products is increasing because of their antimicrobial activity against many human as well as animal pathogens [56]. In clinical and foodborne pathogenesis bio film associated infection is known

as a trigger to chronic diseases, food spoilage. Even dairy and refrigerated food spoilage was also created by the bacterial bio film. [57, 58]. Developed studies are demonstrating that there is a biological rationale between quorum sensing and bio film which work on a coordinate manner leading to spoilage. [59]. Adhesion and bio film formation are also important virulence factors in *S. aureus* since they promote the colonization of food environments [60]. In fact, bio films formed on food processing surfaces enhance the tolerance to disinfectants, thereby increasing the risk of cross-contamination of food. Of note, bio film formation by *S. aureus* can be enhanced by some processing conditions used in the food industry, such as a suboptimal growth temperature or the combined presence of salt and glucose [61].

## Conclusion

The contamination level of food contact surfaces with *S. aureus* suggests that the handling of livestock as well as cleaning and disinfection of food industry facilities must be improved. In this respect, it must be pointed out that all isolates showed the potential ability to form bio films, which may allow them to adhere to different food contact surfaces. These bio films could be a potential source of food contamination, likely due to an unsatisfactory application of disinfectants against bio film-associated cells. In addition, food industry surfaces seemed to be a reservoir for other food pathogenic bacteria as well as for some food spoilage microorganisms coexisting with *S. aureus*. In turn, the presence of *S. aureus* in mixed-species bio films could also enhance the colonization and persistence of this bacterium in the food environment. These results therefore point toward the need to improve hygiene conditions during the production of food.

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