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Antibacterial Effects of Fenugreek, Wheat and Hot Red Pepper Seeds and Their Germs Extract on Inhibiting *Staphylococcus aureus* and *Enterobacter cloacae* Growth

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Authors' contributions

This work was carried out in collaboration between both authors. Authors NHY and MAS helped in data curation, method preparation, investigation, literature searches and statistical interpretation. Author NHY supervised the study. Authors NHY and MAS wrote the original draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The work investigated the effects of plant seeds such as fenugreek (*Trigonella foenum-graecum* L.), wheat grains (*Triticum aestivum* L.), and hot red pepper (*Capsicum annuum* L.) which are traditionally used as natural food preservatives or at least to minimize the used concentrations of artificial preservative, meantime to serve as antibacterial agents against certain positive and negative gram bacteria such as *Staphylococcus aureus* ATCC 29213 and *Enterobacter cloacae* Enk1 LT592256 and the effectiveness of using germinated seeds in reducing bacterial growth.
Study Design: The experiments were designed as one way completely randomized design.
Place and Duration of Study: The experiments were carried out at the Regional Center for Food and Feed (RCFF), Agricultural Research Center, and Faculty of Agriculture Saba Basha, Alexandria University, Egypt.

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Methodology: The living bacterial cells in each treatment was estimated after 5 days of incubation at 25°C the total viable bacterial counts were determined using the plate count agar method. Microbial numbers in all samples were determined using serial dilutions also, determine the total flavonoids, antioxidant activity, and total phenol contents of the tested seeds and their germs.

Results: Our results demonstrated that the maximum number of *S. aureus* bacteria in 10^{-1} on wheat germinated seeds extract at 1000 µl that is 290 CFU and the maximum number of *E. cloaceae* was 370 CFU was found in wheat non-germinated seeds at 500µl concentration, however, the highly inhibitory concentration of *S. aureus* 1000 µl was realized using non-germinated red pepper seeds extract (97.9%) and (92.9%) in the case of *E. cloaceae* bacteria. The maximum inhibition percentage was found with red pepper non-germinated seeds for (*S. aureus*) it was (97.9%) and for the (*E. cloaceae*) was (92.9%) under the concentration 1000 µl. The results showed that germs extract was less effective as an antibacterial agent than seed extract. *S. aureus* was more susceptible than *E. cloacae*. The use of hot red pepper seeds extracts then fenugreek extract was highly effective in reducing the growth of both the tested bacteria.

Conclusion: Hot pepper and fenugreek extracts can be used as natural alternative preventives to control food poisoning diseases. Non Germinated seeds are most effective against studied bacteria growth.

Keywords: Seed extracts; germinated seed extracts; antioxidant activity; Staphylococcus aureus; Enterobacter cloacae.

1. INTRODUCTION

Egyptian pharaoh is using fenugreek to bread and many other foods are safe and healthy much longer. After that, the ancient Greek people use herbs and spices for saving food and medical uses. Now, the prevention of food spoilage and food poisoning pathogens is usually achieved using chemical preservatives that threaten human health because of their chemical traces hazardous in food chains, so, the imperative need to find potentially effective, healthy safer and natural substitute preservatives are increased [1]. Lipid Oxidation is one of the principal factors of chemical spoilage in food goods, rancidity and deterioration of the quality, color, flavor, texture and safety of the products [2,1]. Alternatively, microbial activity is a primary mode of deterioration of many victuals and is often liable for the loss of quality and safety [3]. There is growing interest in using natural antibacterial compounds such as plant extracts of herbs and spices for the preservation of foods because these extracts own a characteristic flavor and occasionally show antioxidant activity and antimicrobial activity [4]. Plants have formed the basis for traditional medicine systems and have been used for the last century in countries such as China and India, which play a vital role in health care. Various plants (whole or some parts) are known to be helpful used for human health support, one of these benefits, is the capacity of some plant parts exerting pharmacological activities like, analgesics, diuretics, antispasmodic, antimicrobial activity [5]. The antioxidant features of the plant material are due to the presence of many active phytochemicals such as, vitamins, flavonoids, terpenoids, carotenoids, coumarins, curcumins, lignin, saponin and sterols 'etc.' [6,7] phytochemicals often used for treating various illnesses [8]. Fenugreek (*Trigonella foenum-graecum L.*), is an annual legume crop mainly grown for use as a spice in many world [9] consider one of the oldest medicinal plants recognized in history [10,11]. Fenugreek seeds include lysine and L-tryptophan rich proteins, mucilaginous fiber and some chemical ingredients such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects [12]. *T. foenum-graecum* have anti-diabetic, anti-fertility, anticancer [12], anti-microbial [13], anti-parasitic, and hypocholesterolaemic effects [14] contains volatile oils and alkaloids are venomous to parasites, as antimicrobial [15].

Chili (*Capsicum annuum* L.) peppers are used worldwide in foods for their pungent flavor, aroma, and to prolong food spoilage its fruit contains a large diversify of antioxidant vitamins, especially vitamin A and C, capsaicin [16,17]. Hot cultivars are rich in capsaicinoids was used for its pharmacological properties [11]. Capsaicin is an alkaloid (capsaicinoid) found in the placental tissues that hold the seeds in chilies, tissue extracts from several different *C. annuum* var. jalapeno have inhibited the growth of species of *Bacillus, Clostridium, Pseudomonas,* *Listeria, Salmonella, Staphylococcus,* and *Streptococcus* [18] and antimicrobial component especially against *Listeria monocytogenes* [19] were determined the best method for extraction from *C. annum var. jalapeno* extract which ranged between 20 and 30 min, contained the most antimicrobial activity, especially against *L. monocytogenes*.

Capsaicin (8-methylN-vanillyl-6-nonenamide) and dihydrocapsaicin contribute almost 90% of the pungency [20,21]; however, other studies report that high concentrations of commercial capsaicin were inhibitory against *Bacillus subtilis*, specifically [22]. Carotenoids extracted from dried peppers were evaluated for their antioxidant, analgesic, and anti-inflammatory activities [23]. Kalia et al., [24], evaluated the possibility of capsaicin behaves as an inhibitor of the NorA efflux pump of *Staphylococcus aureus,* [25] studied the best antibacterial activity of chili sauce had against *Salmonella enterica*. Some concentrations have a weaker inhibition effect on growth of bacteria. Also, chili sauces have weaker antibacterial effect against *Escherichia coli* and *Bacillus thuringiensis.* [22]. [26] indicated the inhibitory effect of the extract of *Capsicum annuum* bell pepper type against *Salmonella typhimurium* and *Pseudomonas aeruginosa.*

Wheat germ is one of the most potential and excellent sources of vitamins, minerals, fiber and proteins [27]. Padalia et al., [28] reported that wheat extract has a high content of bioflavonoids that may add toward antimicrobial effects [29]. Wheat germ is a food by-product with high nutritional value, especially as a concentrated source of dietary fiber and essential fatty acids, but its incorporation into the diet has been rare up to now. Matteuzzi et al., [30] showed that ingestion of commercial wheat germ modifies the human colon microflora by lowering Gram-negative bacteria such as coliforms, while increasing potentially health promoting bacteria, such as bifidobacteria and lactobacilli. However, existing studies reporting wheat germ are not sufficient to validate a causal relationship, especially due to the limited number of participants included.

Germinated seeds have several beneficial properties than un-germinated seeds. They are a well resource of essential amino acids mainly leucine, lysine and tryptophan. The general hypothesis that germination improves in vitro protein digestibility, as well as fat absorption capacity [31,32]. The germination process decreases the levels of total unsaturated fatty acids, total lipid, triglycerides, phospholipids and unsaponifiable matter, while those of saturated fatty acids are increased [33]. Soaking, germination and roasting enhanced the total phenolic content and antioxidant activity of fenugreek seed flour, also the antioxidant activity of the extracts of soaked, germinated and roasted fenugreek seed was higher than the raw fenugreek seed flour however, the phytic acid content significantly ($P < 0.05$) decreased compared to raw seeds [33,34]. Dixit et al., [35] revealed that the significant high antioxidant activity in germinated fenugreek seeds may be partly due to the presence of flavonoids and polyphenols, they concluded that fenugreek seeds on the seventh day of germination can be used as nutraceutical for the administrative of oxidative stress-related disorders. also, with germinated fenugreek seeds with no toxicity for human and diabetic patients after 24 weeks of daily usage [36].

Enterobacter cloacae is a gram-negative bacterium that belongs to the family Enterobacteriaceae is responsible for the vast majority of Enterobacter infectious of humans can cause death rapidly if untreated. They can be both aerobic and anaerobic. *Enterobacter cloacae* caused wound respiratory [37], and urinary tract infection. ESBL-bacteremia (extended-spectrum β-lactamase producing bacteria) [38]. In addition, *E. cloacae* was resistant to ampicillin and first and second generation of cephalosporins [37,38].

Staphylococcus aureus is considered the most effective food-borne bacterial pathogen that has ever evolved. Its metagenome contains 10s of genes encoding staphylococcal enterotoxins, which are responsible for the clinical symptoms associated with staphylococcal food poisoning it is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus* (Staph) bacteria [39]. Antibiotic resistant *S. aureus* and methicillin-resistant [39,40].

Our main scientific works was based on investigating the role of certain plant seeds and their germs extracts as antibacterial agents against *S. aureus* and *E. cloacae* growth as examples of gram positive and negative grams bacteria. The target throws this study was to reduce food spoilage, to minimize the add amounts of artificial preservatives and preserve food during its processing, handling, storage and even when it is eaten. The specificities of our work were built on investigating the germs usage in food processing after studying their phytochemicals properties.

2. MATERIALS AND METHODS

2.1 Preparation of the Tested Seeds

Dried mature seeds of fenugreek, seeds of hot red pepper fruit and whole wheat grains were purchased from a local supermarket in Alexandria, Egypt. These seeds were purified from foreign material, then gently washed with sterilized water the non-germinated seeds were sprayed 20 g each with alcohol 70% and oven dried for 72 hrs. at 40°C to keep all seed components safe from any degradation [15].

2.1.1 Germination process

Fenugreek, hot red pepper and wheat seeds were purchased from a local supermarket in Alexandria, Egypt 20 g of seeds were sprayed with 70% alcohol for surface sterilization, each species of seed was gently placed between two sterilized watman filter paper imbibed with sterilized water. Petri dishes were incubated at 25°C for one week. The germinated seeds were dried for 72 hrs. at 40°C to keep all seed components safe from any degradation. After dryness each seed were finely ground and kept at 4°C for further analysis.

2.2 Bacterial Strains and Culture Media

Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) was brought from Microbiology Dept (CAICC), Faculty of Agriculture, Cairo University. The second strain Gram-negative *Enterobacter cloacae* (Enk1 LT592256) bacteria was kindly and generously offered by Dr. Amany Shams, Department of Plant Pathology, Bacterial Lab, Faculty of Agriculture, Alexandria University cultures preparation and identification according by Bacon et al. [19].

2.2.1 Inoculum preparation

Each bacterial strain was sub-cultured in Brain Heart broth for 24 h. At 25°C were harvested individually by centrifugation at 600 rpm for 10 min and then washed twice with a phosphate buffer and turbidity was adjusted to an optical density (O.D.) of 0.85 at 600 nm using a spectrophotometer, 200 µl. The cell suspension for both bacterial strains were inoculated into nutrient agar and incubated at 25°C for 48 h. *Staphylococcus aureus* according to Youssef et al. [41] and *Enterobacter clocae* after applying standard method using a serial dilution according to Arashisar et al., [42] the plates count was recorded as serial dilutions.

2.3 Testing the Effects of the Seeds and Their Germs or Sprouts Extracts on the Bacterial Growth

The experiment was conducted by placing each treated infected and infected untreated plate group were grown using specific broth media for each bacterial strain. At the end of the incubation period, samples were taken for bacterial viable count and the MIC (minimum inhibitory concentration) of each treatment was determined.

2.3.1 Viable plate count

The effectiveness of each treatment was estimated by determination of living bacterial cells in each treatment after 5 days of incubation at 25°C on plate count agar. Each treatment 20 g was serially diluted after preparation as mentioned before, total viable bacterial counts were determined using the plate count agar method [42]. The plates were incubated for 48 hrs. at 25°C for total viable counts of *E. cloaceae* and *S. aureus* all counts were expressed as log10 cfu/ml and performed in triplicate

$$Number\ of bacteria/ml\ =\ \frac{number\ of\ colonies\ (CFUs)}{dilution\ X\ amount\ plated} =$$

The MICs were determined according to Wiegand et al. [43] with certain modifications among the two tested seeds and germs extract concentration because pilots tested concentrations less than 500 µl (100 and 200) are not realized good inhibitory results for the two tested bacteria.

2.4 Preparation of Extracts

Stirring 1 g of dry plant material powder of seeds or/ and their germs seeds with 10 mL of pure ethanol for 30 minutes at 120 rpm using a magnetic stirrer plate. The extracts were then maintained for 24 hours at 4°C, filtered through Whatman No. 4 filter paper, and evaporated under vacuum to dryness and stored at 4°C, until analyzed.

2.4.1 Determination of total phenolic content

Antioxidant activity of different extracts was determined by estimating TPC following the method adopted by Musa et al. [44]. Approximately 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent was added to 100 µL fenugreek seeds extracts. The samples were set aside for 5 min and 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance of sample was then taken at 765 nm using a spectrophotometer after 2 hrs. The calibration curve of Gallic acid was used for the estimation of sample activity. The result was recorded in terms of mg of Gallic acid equivalents per 100 g of dry powder (mg GAE/100 g of dry powder) DPPH.

2.4.2 Determination of total flavonoid content

The total flavonoid content was measured by a colorimetric assay, based on the method with certain modification described by (Shi et al. 2012 and Zhao et al. [45]). The total flavonoid content (i.e., three replicates per treatment) was expressed as mg catechin equivalent (CE)/g DW through the calibration curve with catechin. The calibration curve range was 50–500 mg/mL.

2.4.3 Evaluation of the total antioxidant capacity

The antioxidant activity (DPPH assay) was measured by radical scavenging ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot of the sample extract was combined with 1 mL of a reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) using the method described by Asnaashari et al. [46] with some modifications. The tested samples were added to tubes containing 5.9 ml of 0.1 mM methanolic DPPH solution. The reaction mixtures were shaken and kept in the dark. Finally, the decrease in absorbance at 517 nm was measured. The results were calculated using the following equation.

$$\%\text{Radical scavenging capacity} = (AB-AA)/AB*100$$

where: AB = absorption of blank sample and AA = absorption of sample solution

2.5 Statistical Methods

Data of the experiment were statically analyzed in one way completely randomized design and these data were subjected to statistical analysis using Costat computer package (CoHort Software, Berkeley, CA, USA). One-way ANOVA was used and the comparison between the resulting data was done using the least significant difference (LSD) according to Duncan's Multiple Range test was used to compare the treatment mean values with confidence coefficient 5% according to McDonald [47]. Each treatment was replicated three times.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Seed Extracts

The effects of fenugreek, hot red pepper and wheat seeds and their sprouts or germ extracts on the tested *staphylococcus aureus* and *Enterobacter cloacae* growth as alternative to artificial food preservatives were investigated. Our results as illustrated in Fig. 1 showed different phytochemical profiles of seeds and their germs extracts, our data summarized the phytochemical screening of chemical constituents such as total phenol content (mg /g DW), total flavonoids (mg /g DW) and antioxidant activity (%). The represented phenols, flavonoids and antioxidant activity were high in both fenugreek (149.64,11.32 52.9) and (186.16, 200.18 AND 200.18) and hot pepper seeds (166.85,121.5 ,15.86) and (337.66,206.18 ,46.6) in both cases of germinated and non-germinated) respectively while wheat seeds show the lowest phytochemical content. Flavonoid seems to be equal in fenugreek and hot pepper non-germinated seeds, but low in wheat-germinated seeds. Alternatively, hot pepper shows the highest presence of total phenolic content also, the antioxidant activity gives the best screening in fenugreek non-germinated seeds. Phytochemical analysis also revealed composites on corn and defatted soy flour as a good source of phenolics (11.8- 17.87 µg GAE/mg sample), flavonoids (21.6-106.4 µg QE/ mg sample), and antioxidant. Our findings are in agreement with those of Ammar et al.,[48] and Gunani et al., [49] who revealed that flavonoids, antioxidant activity and total phenol contents are highest in non-germinated seeds than hypocotyls and cotyledons as germs ,but our results are opposite to those of Bhatt and Gupta [50], which indicated that wheat flour treated with sorghum, buckwheat, chickpeas, sprouted wheat and sprouted barley and screening has enriched nutritional and phytochemical profile, which makes them a good source of antioxidants. On the other hand, some studies have shown that fenugreek seed (FS) extracts were prepared using different extraction solvents revealed the extractability of bioactive compounds the highest phenolic (156.3 mg GAE/g) and flavonoid (38.5 mg CE/g) contents were obtained from water-germinated seeds [35,51]. In contrast, our present study shows highly phenolic contents (200.18 and 149.64 mg/g DW) and flavonoid contents (186.16 and 11.32 mg/gDW) as compared with non-germinated and germinated seeds, respectively. While our data deals with (Pająk et al., 2019) indicated that, significant ($p < 0.05$) changes in the individual mineral composition of the seeds, improvement of their antioxidant properties, as well as increase in levels of individual phenolic compounds were found after seed germination. Also, [52,23] show that peppers are regarded as a good source of nutrients and phytochemicals, such as ascorbic acid, carotenoids and phenolic compounds, with well-known antioxidant properties and potential health benefits.

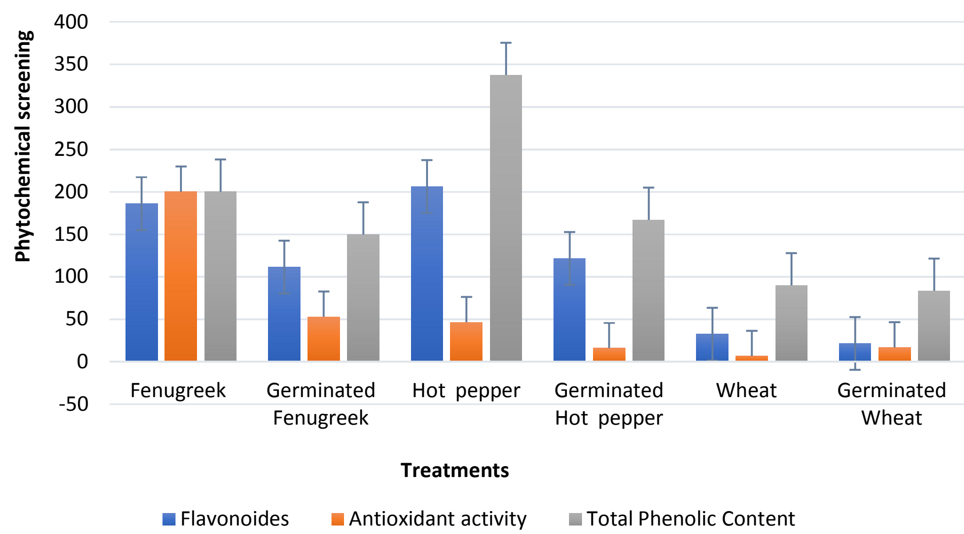

Fig. 1. Phytochemical screening of seed extracts, total phenol content (mg /g DW), total flavonoids (mg /g DW) and antioxidant activity (%) in germinated and non-germinated seeds of Fenugreek, Hot pepper, and Wheat. The values are presented as the mean of three replicates ± the standard deviation. The data marked with different letters share significance at *p <0.05*

Table 1. Effect of tested treatments on *Staphylococcus aureus* growth (cfu)

Treatments	Conc. Per Microliter	Serial dilutions CFU/ml					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Staphylococcus aureus	Control	430^{a}	415^{a}	388^{a}	378^{a}	248^{a}	238^{a}
Fenugreek seed extract	500	268^{e}	112^{i}	96^{i}	53^{i}	35^{j}	30^{j}
Fenugreek seeds extract	1000	169^{j}	116^{h}	85^{j}	78^{j}	64^{h}	32^{k}
Fenugreek germinated seeds	500	208^{hi}	146^{g}	138^{h}	93^{h}	71^{g}	40^{h}
Fenugreek germinated seeds	1000	156^{k}	60^{j}	53^{k}	51^{k}	49^{l}	26^{j}
Red pepper seeds extract	500	207^{i}	48^{k}	38^{l}	44^{l}	35^{j}	19^{l}
Red pepper seeds extract	1000	113^{l}	31^{l}	14^{m}	12^{m}	9^{k}	5^{m}
Red pepper germinated seeds extract	500	227^{f}	235^{e}	220^{e}	149^{e}	117^{e}	122^{d}
Red pepper germinated seeds extract	1000	317^{b}	279^{b}	276^{b}	240^{b}	158^{c}	133^{c}
Wheat seeds extract	500	217^{g}	180^{f}	150^{g}	125^{g}	65^{h}	47^{g}
Wheat seeds extract	1000	209^{g}	180^{f}	168^{f}	162^{d}	105^{f}	56^{f}
Wheat germinated seeds extract	500	270^{d}	240^{d}	229^{d}	226^{c}	213^{b}	155^{b}
Wheat germinated seeds extract	1000	290^{c}	275^{c}	260^{c}	140^{f}	129^{d}	97^{e}
$LSD_{0.05}$		1.678	1.635	1.678	1.678	1.678	1.762

**Data are means of three replicates. Which are exhibited in a column followed by the same letter do not differ significantly at P=0.05. Conc.: concentration; LSD: the least significant difference*

3.2 Total Count and Growth Rates of Each Tested Bacteria

Three seeds species Fenugreek, hot pepper and wheat and their germs under two concentrations (500 µg/ml and 1000 µg/ml) of their ethanolic extract were investigated to evaluate their antibacterial activity against food poisoning bacteria including one strain of Gram-positive bacteria (*S. aureus*) and one strain of Gram-negative bacteria (*E. cloaceae*) determined by serial dilution then the total plate count method. Evaluation of the antibacterial activity of these plant extracts is recorded in Tables 1 and 2. These results demonstrated that the maximum number of *Staphylococcus aureus* bacteria in 10^{-1} on wheat germinated seeds extract at 1000 µl is 290 cfu and the maximum number of *Enterobacter cloaceae* (370 cfu) were found in wheat non-germinated seeds at 500 µl concentration. However, the highly inhibitory concentration of *S. aureus* 1000 µl was realized using non-germinated red pepper seeds extract (97.9%) and (92.9%) in the case of *Enterobacter cloaceae* bacteria. On the other hand, *S. aureus* bacteria recorded the lowest number at red pepper non-germinated seeds with 1000 µl concentration (9 and 5 cfu) at $10^{-5}$and 10^{-6} dilutions, respectively. All plant extracts were potentially effective in reducing microbial growth of food poisoning bacteria with variable potency. One the other hand non-germinated red pepper seed extract with ~~the~~ ~~a~~t concentration of 1000 µl/ml had the most inhibition with all dilutions while extract of wheat germinated seeds had the low effective with all concentrations. Fenugreek seeds extract even germinated or not-germinated showed the second effective antibacterial extract against both of *S. aureus* or and *E. cloaceae*. Table 2 showed no significant differences between 500 µl and 1000 µl seed concentration, they were (30 and 32 cfu) respectively at 10^{-6} dilution also, *Enterobacter cloaceae* had a significant impact between germinated and non-geminated fenugreek seeds under 500 and 1000 µl extract concentrations. Otherwise, the results with *Enterobacter cloaceae* revealed that red pepper non-germinated seeds had the lowest bacterial growth under 1000 µl concentration, it was 15 cfu in 10^{-6} dilution. Although red pepper germinated seeds and wheat non-germinated seeds recorded the same *E. cloaceae* count (207 cfu) under 500 µl seed concentration in 10^{-6} dilution. The results of screening plant extracts for antimicrobial activity were summarized in Tables 1 and 2. It was found that some plant extracts had antibacterial activities in different degrees against *S. aureus,* there is a significant difference between non-germinated and germinated fenugreek seed extract appeared clearly in the high dilutions 10^{-5} and 10^{-6} under 500 µl concentration it was (35, 71 and 30,40 cfu). In contrast the red pepper non-germinated seeds had a highly significant impact compared to germinated seeds under the same concentration. As general non-geminated seeds for the three plants appeared to have inhibitory effects on the *S. aureus* bacteria and *E. cloaceae* too except for wheat seeds, the germinated seeds showed more effective than non-germinated seeds. Alternatively, our results demonstrated that the plants extract concentration 1000 µl had a significant effect compared to 500 µl concentration. Moreover, the same trend was found between serial dilutions for the both strains of bacteria. These data accordance with those of Tajkarimi et al., [53]; Dixit et al., [35] indicated that most herbs and spices extract antimicrobial activity against different (bacteria, yeasts, and molds). Phenolic compounds obtained from herbs and spices show biological activity and can be potentially used as food preservatives [54]. Our results were emphasized by Sharma et al., [55] who showed that fenugreek leaves, seeds and stem extract (Methanol, Acetone and aqueous extract) had antimicrobial activity against *E. coli* and *Staphylococcus,* which were determined by the well diffusion method. Furthermore, fenugreek germinated seeds are considered to be more beneficial than dried seeds [35]. Moreover, the extract of *Capsicum annuum* bell pepper prevented the growth of *Salmonella* typhimurium at 1.5 mL/100 g concentration in minced beef [19]. Meanwhile, extracts of several different *C. annuum* varieties have inhibited the growth of species of *Bacillus, Clostridium, Pseudomonas, Listeria, Salmonella, Staphylococcus, and Streptococcus*. Extract from jalapeno fruit, specifically, has inhibited *Streptococcus pyogenes*, *Listeria monocytogenes*, *Clostridium sporogenes*, and *Clostridium tetani* [18,19]. Chili peppers are used worldwide in foods for antimicrobial activities against numerous human pathogens (Omolo et al., 2017). Those results agree with both bacterial counts inhibited by red pepper concentration 1000 µl they were (5 and 15 cfu) for *S. aureus* and *E. cloaceae,* respectively, in 10^{-6} dilution. Our present study deals with data obtained from [56] study who evaluated that *Trigonella foenum graecum* (Fenugreek), in three concentrations (50 mg/ml, 100 mg/ml, and 200 mg/ml) have antimicrobial activities against two gram-positive bacteria:

Staphylococcus aureus, *Streptococcus sp*., and two gram-negative bacteria: *Escherichia coli*, *Proteus sp*. [57] reported the same results against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella spp*.

3.3 Efficacy Ratio Percentages (ER %) of Each Tested Extract against the Two Tested Bacteria

The maximum inhibition percentage was found with red pepper non-germinated seeds for (*S. aureus*) it was (97.9%) and for the (*E. cloaceae*) was (92.9%) with the same treatment under the high seed's concentration 1000 µl. Furthermore, the lowest inhibitory seed extract was observed with wheat germinated seeds against *S. aureus* was (59.2%) while *E. cloacae* showed different results, the lowest inhibition efficacy percentage is (1.4%) was realized using red pepper-germinated seeds at 500 µl and wheat non-geminated seeds at 500 µl concentration, respectively, according to Fig. 5 and Table 3. Our results are closely related to those of Saha et al., [58] who reported that the antimicrobial properties of wheat grains differed according to their varieties. Additionally, our findings are not matching with those of Sharma et al., [55], who were observed that an extract of *Trigonella's* leaves, stem and seeds methanol extract of leaves had a maximum inhibition against *E. coli* and *Staphylococcus.* The inhibitory effects were differed maybe due because plants and herbs have classes of phytochemicals according to their species, the time of collection, cultivation season, geographic origin and the used solvent [8,15,58]. According to Molina-Torres et al., [22], capsaicin (pure), had a strong inhibitory effect on *B. subtilis* starting from 25 µg/ml (minimum concentration assayed) also, [24], evaluated the possibility of capsaicin acting as an inhibitor of the NorA efflux pump of *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of ciprofloxacin was reduced 2 to 4-fold in the presence of capsaicin.

3.4 Minimum Inhibitory Concentrations (MICs) of the Tested Seeds and Germs Extracts against the Two Tested Bacteria at all Used Serial Dilutions

According to Figs. 2 and 3 The best MIC was realized by red pepper seed extract in both tested bacteria followed by fenugreek seeds extract at a concentration 500 µl/ml in case of *S. aureus* and at concentration 1000 µl/ml in case of *E. cloacae*. [59,38] indicated the *E. cloacae* was more resistant to these extracts as antibacterial agent because they carry plasmids encoding resistance to multiple antimicrobial

Table 2. Effect of tested treatments on *Enterobacter cloaceae* growth (cfu)

Treatments	Conc. Per Microliter	Serial dilutions CFU/ml					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Enterobacter clocae	Control	450 ^{a}	434 ^{a}	374 ^{a}	341 ^{a}	313 ^{a}	210^{a}
Fenugreek seed extract	500	221 ^{j}	211 ^{k}	198 ^{k}	117 ^{l}	66 ^{l}	96 ^{g}
Fenugreek seeds extract	1000	126 ^{k}	100 ^{l}	86 ^{m}	74 ^{m}	35 ^{m}	31^{i}
Fenugreek germinated seeds	500	301 ^{g}	226 ^{i}	221 ^{i}	212 ^{h}	200 ^{g}	189 ^{d}
Fenugreek germinated seeds	1000	317 ^{f}	289 ^{e}	280 ^{e}	252 ^{e}	224 ^{e}	164 ^{e}
Red pepper seeds extract	500	287 ^{h}	263 ^{g}	208 ^{j}	193 ^{j}	160^{j}	23 ^{j}
Red pepper seeds extract	1000	275 ^{i}	220 ^{j}	170 ^{l}	155 ^{k}	107 ^{k}	15 ^{k}
Red pepper germinated seeds extract	500	330 ^{e}	292 ^{d}	286 ^{d}	276 ^{d}	240 ^{b}	207^{ab}
Red pepper germinated seeds extract	1000	274^{i}	262^{j}	253 ^{f}	248 ^{f}	234 ^{c}	193 ^{c}
Wheat seeds extract	500	370 ^{c}	350 ^{b}	315 ^{c}	301 ^{b}	230 ^{d}	207^{ab}
Wheat seeds extract	1000	366 ^{d}	320 ^{c}	312 ^{b}	283^{c}	209 ^{f}	206 ^{b}
Wheat germinated seeds extract	500	329 ^{e}	260^{h}	244 ^{g}	200 ^{i}	190 ^{h}	100^{f}
Wheat germinated seeds extract	1000	374 ^{b}	280^{f}	238 ^{h}	218 ^{g}	162 ^{i}	80 ^{h}
$LSD_{0.05}$		2.883	2.825	2.825	3.183	3.183	2.805

**Data are means of three replicates.*

Which are exhibited in a column followed by the same letter do not differ significantly at P=0.05. Conc.: concentration;

LSD: the least significant difference

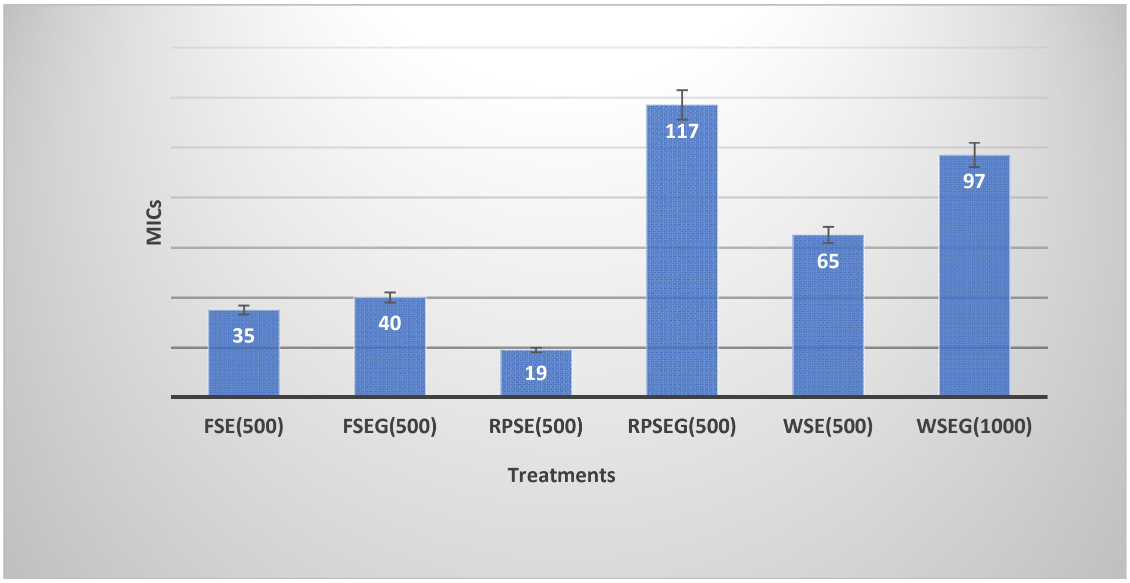

Fig. 2. Minimum inhibitory concentrations (MICs) of the tested seeds and germs extracts against *S. aureus* growth with most effective serial dilutions. Fenugreek seeds and germs extracts (FSE and FSEG) Red pepper seeds and sprouts extracts (RPSE and RPSEG), and wheat seeds and germs extracts (WSE and WSEG) successively

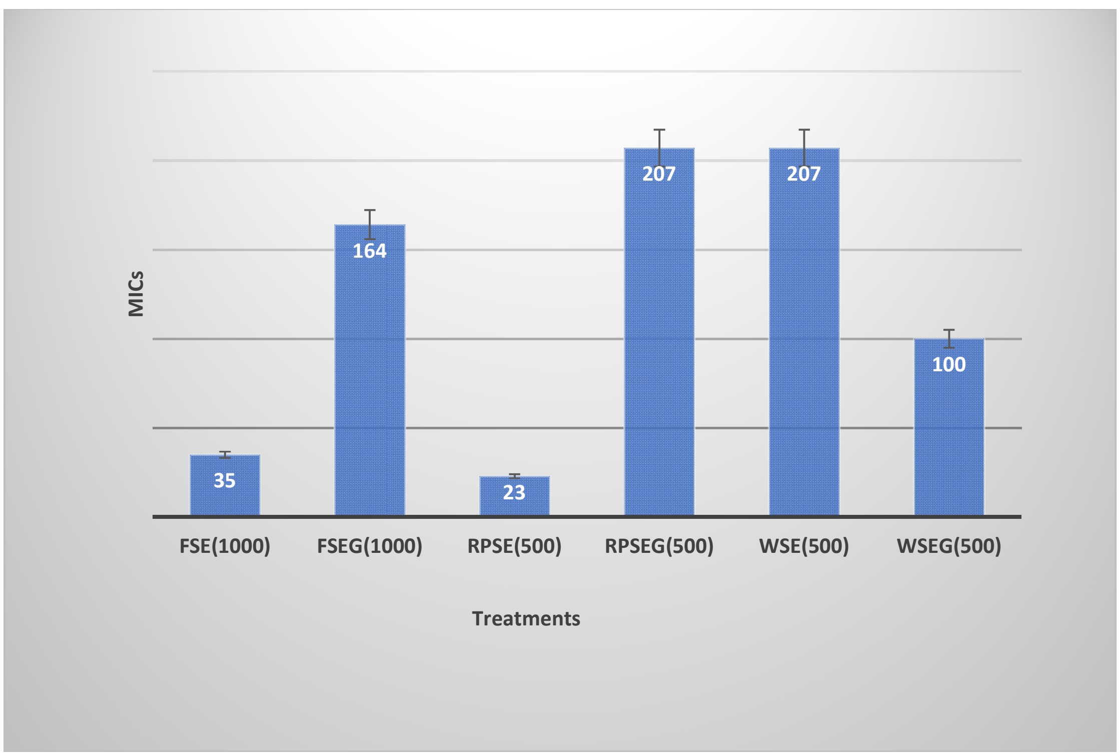

Fig. 3. Minimum inhibitory concentrations (MICs) of the tested seeds and germs extracts against *E. cloacae* growth with most effective serial dilutions. Fenugreek seeds and germs extracts (FSE and FSEG) Red pepper seeds and sprouts extracts (RPSE and RPSEG), and wheat seeds and germs extracts (WSE and WSEG) successively

Table 3. Best efficacy ratio percentage realized by the used extracts against the tested bacteria as total colonies forming unites number (cfu)

Tested bacteria	Control	Fenugreek		G. Fenugreek		Hot Red pepper		G.Hot red pepper		Wheat grains		G. Wheat grains	
		Concentrations											
		(500 µl)	(1000 µl)	(500 µl)	(1000 µl)	(500 µl)	(1000 µl)	(500 µl)	(1000 µl)	(500 µl)	(1000 µl)	(500 µl)	(1000 µl)
S. aureus	238	30	32	40	26	19	5	122	133	47	56	155	97
RF %		87 .39	86.56	83.19	89.08	92.02	97.90	48.74	44.12	80.252	76.47	34.87	59.24
E. clocae	210	96	31	189	164	23	15	207	193	207	206	100	80
RF%		54.29	85.24	10.0	21.91	89.05	92.86	1.43	8.10	1.43	1.91	52.38	61.91

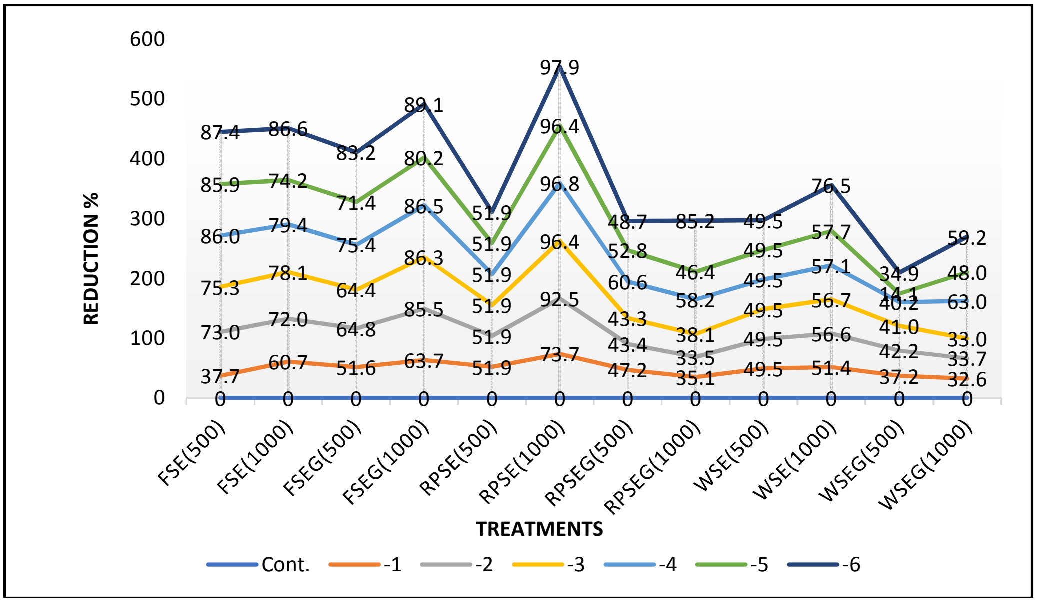

Fig. 4. Efficacy ratios of tested extracts in reducing *Staphylococcus* aureus growth in Fenugreek seeds and germs extracts (FSE and FSEG) Red pepper seeds and sprouts extracts (RPSE and RPSEG), and wheat seeds and germs extracts (WSE and WSEG) successively. The values are presented as the mean of three replicates ± the standard deviation

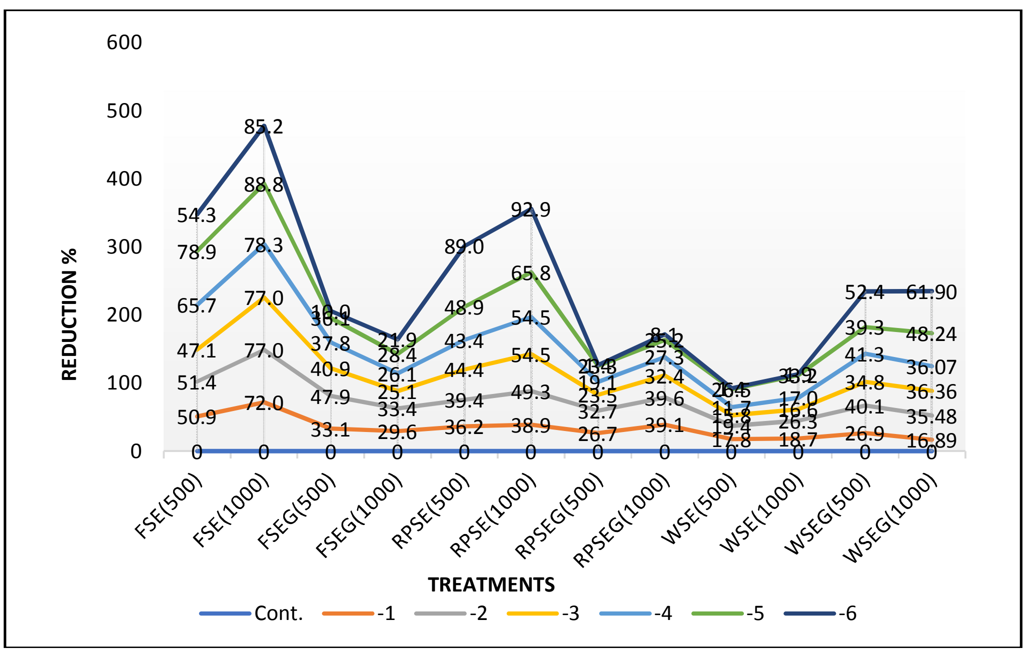

Fig. 5. Efficacy ratios of tested extracts in reducing *Enterobacter cloaceae* growth in Fenugreek seeds and germs extracts (FSE and FSEG) Red pepper seeds and sprouts extracts (RPSE and RPSEG), and wheat seeds and germs extracts (WSE and WSEG) successively. The values are presented as the mean of three replicates ± the standard deviation

agents. According to all our resulted data which are exhibited above, we can notice that the antimicrobial properties of our tested seeds and germ extracts have been explained by the chemical association of active substances; however, the activity of their extracts is highly related to their antibacterial efficacy and species of the tested bacteria for instance, wheat germs and other seeds extracts are an active inhibitory agent against *S. aureus* as gram positive than *E. cloacae* as gram-negative bacteria. These findings are relatively in harmony with those of Srinivasan, [60]. Furthermore, our resulting data exhibited clearly that *S. aureus* was less resistant to seeds and germs extracts than *E. cloacae* these findings are highly in agreement with those of Michael, [59] who reported that the virulence and the high resistance *of E. cloacae* against antibacterial agents and drugs may be due to its production of aerobactin that adheres to tissue culture cells and cause mannose- sensitive and hem agglutination , which is possibly the result of type 1 fimbriae expression caused by this bacterium, or *E. cloacae* lacked cell invasion abilities and exhibited low reactive oxygen species (ROS) production in neutrophils [61]. On the other hand, a remarkable notice is registered in our study that is the reduction of total phenol content, flavonoids and antioxidant activities of the tested germs than the non-germinated seeds. Our results are in agreement with [62] who announced that flavonoids and total phenol contents reduced in Hang rice after the germination process but [63] reported that chick pea, white pea and common vetch have highly antioxidant activity, and total contents in sprouts than dormant seeds, so it clear that the antioxidant activity, flavonoids and total phenol contents in germs depends on the species, kind and variety of the seeds.

4. CONCLUSION

Food spoilage is often caused by the growth of many pathogenic bacterial strains. Prevention of food spoilage in food industry and food stuff is mainly based on the application of chemical preservatives. The adverse effects of these chemical preservatives on human health increases the demand to search for potentially effective, healthy safer and natural food preservative. The plant extracts which proved to be potentially effective as (*Capsicum annuum* and *Trigonella foenum-graecum* L) can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications. Germs used in this study are not effective in reducing bacterial food spoilage growth as the non-germinated seeds that is may be due to the lack of certain effective ingredient during germination process.

5. RECOMMENDATION

Using hot red pepper seeds and fenugreek seeds as preservatives in food processing. The usage of germs during the food processing as alternative to their non-germinated seeds was not recommended

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Suhaj Milan. Spice antioxidants isolation and their antiradical activity: A review. Journal of Food Composition and Analysis. Academic Press; 2006. Available:https://doi.org/10.1016/j.jfca..11.005
2. Antolovich Michael, Paul D, Prenzler Emilios Patsalides, Suzanne McDonald, Kevin Robards. Methods for testing antioxidant activity. Analyst. 2002;127(1): 183–98. Available:https://doi.org/10.1039/b009171p
3. Negi Pradeep. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. International Journal of Food Microbiology 2012;156:7–17. Available:https://doi.org/10.1016/j.ijfoodmicro.03.006
4. Smid EJ, Gorris. Natural antimicrobials for food preservation. In: Rahman MS, Editor. Handbook of Food Preservation. In, 285–308. New York: Marcel Dekker; 1999.
5. Asif Mohammad, Elham Khodadadi. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Journal of Paramedical Sciences 2013;4(3):119–38. Available:https://doi.org/10.22037/jps.v4i3.4648

6. Ushimaru Priscila Ikeda, Mariama Tomaz Nogueira Da Silva, Luiz Claudio Di Stasi, Luciano Barbosa, and Ary Fernandes. Antibacterial activity of medicinal plant extracts. Brazilian Journal of Microbiology. 2007;38(4):717–19. Available:https://doi.org/10.1590/S1517-83822007000400024
7. Vashist H, Dennis Sharma. Pharmacognostical aspects of glycyrrhiza glabra. Asian Journal of Pharmaceutical and Clinical Research. 2013;6:55–59.
8. Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Applied Microbiology. 2000;88(2):308–16. Available:https://doi.org/10.1046/j.1365-2672.2000.00969.x
9. Sulieman A, Ahmed H, Abdelrahim A. The chemical composition of fenugreek (*Trigonella foenum graceum* L) and the antimicrobial properties of its seed oil. Gezira Journal of Engineering and Applied Sciences. 2007;3:52–71.
10. Acharya SN, Thomas JE, Basu SK. Fenugreek, an alternative crop for semiarid regions of North America. Crop Science 2008;48(3):841–53. Available:https://doi.org/10.2135/cropsci2007.09.0519
11. Borel P, et al. Wheat bran and wheat germ: Effect on digestion and intestinal absorption of dietary lipids in the rat. Am. J. Clin. Nutr. 1989;49:1192–1202.
12. Giacomo M, Donato D, Salvatore F. Volatile constituents of sicilian fenugreek (*Trigonella foenum-graecum* L.) seeds. Sciences Des Aliments. 2002;22(3):249–64. Available:https://doi.org/doi:10.3166/sda.22.249-264
13. Pande Kamal Kishore, Lata Pande, Bharat Pande, Atul Pujari, Pankaj Sah, Stuti Sah. Limonene dominates the phytochemistry of *Trigonella foenum-graceum* in nature. Nature and Science. 2011;9(5). Available:http://www.sciencepub.net/nature
14. Al-Habori M, Raman A. Pharmacological properties in fenugreek - the genus Trigonella. G.A. Petropoulos (Ed.), Taylor and Francis, London and New York. 2002; 10:163–82.
15. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science. 2008;86(Suppl 14):E140–48. Available:https://doi.org/10.2527/jas -0459
16. Omolo Morrine. Antimicrobial Properties of Chili Peppers. Journal of Infectious Diseases and Therapy. 2014;02(04). Available:https://doi.org/10.4172/2332-0877.1000145
17. Omolo Morrine, Zen-Zi Wong, Amanda Mergen, Jennifer Hastings, Nina Le, Holly Reiland, Kyle Case, David Baumler. Antimicrobial properties of chili peppers. Journal of Infectious Diseases and Therapy. 2014;02. Available:https://doi.org/10.4172/2332-0877.1000145
18. Bacon Karleigh, Renee Boyer, Cynthia Denbow, Sean O'Keefe, Andrew Neilson, Robert Williams. Antibacterial activity of jalapeño pepper (*Capsicum annuum* Var. annuum) extract fractions against select foodborne pathogens. Food Science and Nutrition. 2017;5(3):730–38. Available:https://doi.org/10.1002/fsn3.453
19. Bacon Karleigh, Renee Boyer, Cynthia Denbow, Sean O'Keefe, Andrew Neilson, Robert Williams. Evaluation of different solvents to extract antibacterial compounds from Jalapeño peppers. Food Science & Nutrition. 2016;5. Available:https://doi.org/10.1002/fsn3.423.
20. Contreras-Padilla Margarita, Elhadi Yahia. "The Evolution of Capsaicinoids during Fruit Development of Three Varieties of Hot Peppers. HortScience. 1997;32:488B–488. Available:https://doi.org/10.21273/HORTSCI.32.3.488B
21. Othman Zeid Abdullah Al, Yacine Badjah Hadj Ahmed, Mohamed Abdelaty Habila, Ayman Abdel Ghafar. Determination of capsaicin and dihydrocapsaicin in capsicum fruit samples using high performance liquid chromatography. Molecules. 2011;16(10): 8919–29. Available:https://doi.org/10.3390/molecules16108919.
22. Molina-Torres JA García-Chávez, Ramírez-Chávez E. Antimicrobial properties of alkamides present in flavouring plants traditionally used in mesoamerica: Affinin and capsaicin. Journal of Ethnopharmacology. 1999;64(3):241–48. Available:https://doi.org/10.1016/s0378-8741(98)00134-2
23. Hernández-Ortega Marcela, Alicia Ortiz-Moreno, María Dolores Hernández-Navarro, Germán Chamorro-Cevallos,

Lidia Dorantes-Alvarez, Hugo Necoechea-Mondragón. Antioxidant, antinociceptive, and anti-inflammatory effects of carotenoids extracted from dried pepper (*Capsicum annuum* L.). Journal of Biomedicine and Biotechnology; 2012.
Available:https://doi.org/10.1155/2012/524019

24. Kalia Nitin, Priya Mahajan, Rukmankesh Mehra, Amit Nargotra, Jai Sharma, Surrinder Koul, Inshad Khan. Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of *Staphylococcus aureus*. The Journal of Antimicrobial Chemotherapy. 2012;67: 2401–8.
Available:https://doi.org/10.1093/jac/dks232

25. Hleba Lukáš, Jana Petrová, Rafal Kordiak, Attila Kántor, Juraj Čuboň, Maciej Kluz, Mohammad Ali Shariati, Miroslava Kačániová. Antibacterial activity of habanero chili sauces against selected pathogenic bacteria. Animal Science and Biotechnologies. 2015;48(1):132–36.
Available:spasb.ro/index.php/spasb/article/viewFile/1958/1900

26. Careaga Mónica, Elizabeth Fernández, Lidia Dorantes, Lydia Mota, Maria Eugenia Jaramillo, Humberto Hernandez-Sanchez. Antibacterial activity of capsicum extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. International Journal of Food Microbiology. 2003;83(3):331–35.
Available:https://doi.org/https://doi.org/10.1016/S0168-1605(02)00382-3

27. Rezq Amr A, Mohamed Y. Mahmoud. Preventive effect of wheat germ on hypercholesteremic and atherosclerosis in rats fed cholesterol-containing diet. Pakistan Journal of Nutrition. 2011;10(5): 424–32.
Available:https://doi.org/10.3923/pjn.424.432

28. Padalia S, Drabu S, Raheja I, Gupta A, Dhamija M. Multitude potential of wheat-grass juice (Green blood): An overview. Chronicles of Young Scientists. 2010;1(2): 23–28.
Available:http://www.jpgmonline.com/article.asp22295186;volume1.Issue,2,Page:23-28.

29. Supria Saha, Islam Zohorul, Islam Sadequl, Md Hossain Shahadat, Islam SM. Shahinul. Evaluation of antimicrobial activity of wheat (*Triticum aestivum* L.) against four bacterial strains-indian journals. Evaluation of Antimicrobial Activity of Wheat (*Triticum aestivum* L.) against Four Bacterial Strains. 2018;20(1): 58–62.
Available:http://www.indianjournals.com/ijor.aspx?target=ijor:skuastjr&volume=20&issue=1&article=010.

30. Matteuzzi D, Swennen E, Rossi M, Hartman T, Lebet V. Prebiotic effects of a wheat germ preparation in human healthy subjects. Food Microbiology. 2004;21(1): 119–24.
Available:https://doi.org/10.1016/S0740-0020(03)00009-1

31. Mansour EH, El-Adawy. Nutritional potential and eunctional properties of heat treated and germinated fenugreek seeds. Lebensmittel Wissenschaft Technologie. 1994;27:568–72.

32. Chalghoumi R, Mabrouki S, Abdouli H, Line JE. Antibacterial activity of fenugreek seeds (*Trigonella foenum-graecum*) crude extracts against a rabbit *Escherichia coli* isolate. 2016;4:139–44.
Available:https://doi.org/10.15413/ajmr.0117

33. Shakuntala Sathyanarayana, Jarpala Pura Naik, Thangaraj Jeyarani, Madineni Madhava Naidu, Pullabhatla Srinivas. Characterisation of germinated fenugreek (*Trigonella foenum-graecum* L.) seed fractions. International Journal of Food Science and Technology; 2011.
Available:https://doi.org/10.1111/j.1365-2621.02754.x

34. Pandey Hemlata, Pratima Awasthi. Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed flour. Journal of Food Science and Technology. 2015;52(2):1054–60.
Available:https://doi.org/10.1007/s13197-013-1057-0

35. Dixit Priyanjali, Saroj Ghaskadbi, Hari Mohan, Thomas P. A. Devasagayam. Antioxidant properties of germinated fenugreek seeds. Phytotherapy Research. 2005;19(11):977–83.
Available:https://doi.org/10.1002/ptr.1769.

36. Khole Swati, Suchandra Chatterjee, Prasad Variyar, Arun Sharma, TPA Devasagayam, Saroj Ghaskadbi. Bioactive constituents of germinated fenugreek seeds with strong antioxidant potential. Journal of Functional Foods; 2014.

Available:https://doi.org/10.1016/j.jff.2013.10.016.

37. Buckle Jane. Infection. In Clinical Aromatherapy, 2015;130–67. Elsevier. Available:https://doi.org/10.1016/B978-0-7020-5440-2.00007-3
38. Annavajhala Medini K, Angela Gomez-Simmonds, Anne Catrin Uhlemann. Multidrug-resistant enterobacter cloacae complex emerging as a global, diversifying threat. Frontiers in Microbiology. 2019;10: 1–8. Available:https://doi.org/10.3389/fmicb.2019.00044
39. Kadariya J, Smith TC, Thapaliya D. *Staphylococcus aureus* and staphylococcal food-borne disease: An ongoing challenge in public health. Biomed Res Int. 2014; 2014:827965. DOI: 10.1155/2014/827965 Epub 2014 Apr 1 PMID: 24804250 PMCID: PMC3988705
40. Costa WLR, Ferreira J. dos S, Carvalho JS, Cerqueira ES, Oliveira LC, Almeida RC de C. Methicillin-resistant *Staphylococcus aureus* in raw meats and prepared foods in public hospitals in Salvador, Bahia. Brazil. J Food Sci. 2015;80(1):M147–M150. Available:https://doi.org/10.1111/1750-3841.1272
41. Youssef NH, Salwa M, Daoud SD, Atwa MAA. Effectiveness of chitosan and some essential oils as maize grain edible coating films on the growth of *Escherichia coli* and *Staphyllococcus aureus* and some mycotoxins produced by Fusarium verticilloides and *Aspergillus flavus*. Journal of Agricultural Chemistry and Biotechenology. 2016;7(2):27.
42. Arashisar S, Hisara O, Kayab M, Yanik T. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. Internat. of Food Microbiol. 2004;97:209–214.
43. Wiegand Irith, Kai Hilpert, Robert E. W. Hancock. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols. 2008;3(2): 163–75. Available:https://doi.org/10.1038/nprot.521
44. Musa KH, Abdullah A, Jusoh K, Subramaniam V. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. Food Anal Method. 2011;4(1):100-107.
45. Zhao LJ, Liu W, Xiong SH, Tang J , huan Lou Z, Ming-xia X, Bo-hou X, Lin LM, Duan-fang L. Determination of total flavonoids contents and antioxidant activity of ginkgo biloba leaf by near-infrared reflectance method. International J. of Analitical Chemistry. 2018;2018(Article ID 8195784). Available:https://doi.org/10.1155/2018/8195784.
46. Asnaashari M, Farhoosh R, Sharif A. Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of kilka fish oil and its oil-in-water emulsion. Food Chemistry. 2014;159:439–44.
47. McDonald John H. Handbook of biological statistics - paired t–Test. Sparky House Publishing. 2014;180–85. Available:http://www.biostathandbook.com/pairedttest.html
48. Ammar MN, Ali MEM, ElNour. Antioxidant activityb, total phenolic, flavonoid and tannin contents of callus and seeds extracts of fenugreek (*Trigonlla foenum-graecum* L.) International Journal of science and Research (IJSR) ISSN (online): 2319-7664; 2012.
49. Gurnani N, Gupta M, Mehta D, Bhupendra KM. Chemical composition,total phenolic and flavonoid contents,and in vitro antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens* L); 2015.
50. Bhatt Supriya Mohan, Rajinder K. Gupta. Bread (composite flour) formulation and study of its nutritive, phytochemical and functional properties. ~ 254 ~ Journal of Pharmacognosy and Phytochemistry. 2015;4(2):254–68.
51. Norziah MH, Fezea FA, Bhat R, Ahmad M. Effect of extraction solvents on antioxidant and antimicrobial properties of fenugreek seeds (*Trigonella foenum-graecum* L.). International Food Research Journal. 2015;22(3):1261–71.
52. Kim Chu Sook, Teruo Kawada, Byung Sam Kim, In Seob Han, Suck Young Choe, Tadao Kurata, Rina Yu. Capsaicin exhibits anti-inflammatory property by inhibiting ikb-a degradation in LPS-stimulated peritoneal macrophages." Cellular Signalling. 2003; 15(3):299–306. Available:https://doi.org/10.1016/S0898-6568(02)00086-4.

53. Tajkarimi Mehrdad, Salam Ibrahim, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010; 21:1199–1218.
Available:https://doi.org/10.1016/j.foodcont.02.003
54. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry. 2004; 11:1451-1460.
Available:http://dx.doi.org/10.2174/0929867043365107
55. Sharma V, Singh P, Rani A. Antimicrobial activity of *Trigonella foenum-graecum* L. (Fenugreek). European Journal of Experimental Biology. 2017;07(01).
Available:https://doi.org/10.21767/2248-9215.100004
56. Al-hussainy Ahmed Dhahir. Evaluation of the antimicrobial (antibacterial and antifungal) activity of ethanolic extracts of some medical plants. 2015;5(22):87–95.
57. Waheed K, Muhammad SK, Shomaila A, Muhammad Z, Izhar U, Ullah S. Antimicrobial activity and phytochemical screening of *Euphorbia helioscopia.* Planta Daninha. 2020;v38:e020213727.
DOI: 10.1590/S0100-83582020380100011
58. Saha S, Islam Z, Islam S, Isalam SMS. Evaluation of antimicrobial activity of wheat (*Triticum aestivum* L.) against four bacterial strains; 2018.
Available:https://www.researchgate.net/publication/326263956
59. Michael S. Donnenberg. Enterobacteriacea in mandell, douglas, and bennett's principles and practice of infectious diseases (Eighth Edition); 2015.
60. Srinivasan Krishnapura. Spices as influencers of body metabolism: An overview of three decades of research. Food Research International. 2005;38(1): 77–86.
Available:https://doi.org/10.1016/j.foodres.09.001
61. Mishra Mitali, Sasmita Panda, Susmita Barik, Arup Sarkar, Durg Vijai Singh, Harapriya Mohapatra. Antibiotic resistance profile, outer membrane proteins, virulence factors and genome sequence analysis reveal clinical isolates of enterobacter are potential pathogens compared to environmental isolates. Frontiers in Cellular and Infection Microbiology. 2020; 10:1–13.
Available:https://doi.org/10.3389/fcimb.00054.
62. Phattayakorn K, Pajanyor P, Wongtecha S, Prommakool A, Saveboworn W. Effect of germination on total phenolic content and antioxidant properties of 'hang' rice. International Food Research Journal. 2016;23(1):406–9.
63. Gharachorloo M, Tarzi BG, Baharinia M. The effect of germination on phenolic compounds and antioxidant activity of pulses. Journal of the American Oil Chemists' Society. 2012;90(3).
DOI: 10.1007/s11746-012-2170-3
Available:https://www.researchgate.net/publication/257730207.

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