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Original Article

In vitro study of antimicrobial activity of some plant seeds against bacterial strains causing food poisoning diseases

Estudo *in vitro* da atividade antimicrobiana de algumas sementes de plantas contra cepas bacterianas que causam doenças de intoxicação alimentar

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Abstract

In this research, some plant seeds powder was evaluated to find their potential effect to rule diseases of food poisoning. Antimicrobial effect of five plant seeds was examined contra *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella. pneumonia* and *Candida albicans* by using well diffusion method. Antimicrobial activity studies revealed high potential activity of plant seeds powder of *Nigella sativa L., cucurbita pepo, Sesamum radiatum, Trigonella foenum-graecum, Linum usitatissimum* with variable efficiency contra tested microbial strains with concentration of 100 mg/ml, except *Sesamum radiatum* scored no effect. The *T. foenum* and *N. sativa* seed powder showed the largest inhibition zone (24-20 mm) contra *K. pneumonia,* followed by *S. aureus* (20-18 mm) and *C. albicans* (15mm) respectively. The five plant seeds powder exhibited bacteriostatic and bactericidal effects with MIC's 20 and MBC 40 mg/ml against *K. pneumonia*, and MIC's 40 and MBC 60 mg/ml against *S. aureus*. The results of this study indicated that plants seeds powder have promising antimicrobial activities and their potential applications in food process. It could be utilized as a natural medicinal alternative instead of chemical substance.

Keywords: plant seed powder, antimicrobial, food poisoning, diseases.

Resumo

Nesta pesquisa, o pó de sementes de plantas foi avaliado para encontrar seu efeito potencial no controle de doenças de intoxicação alimentar. O efeito antimicrobiano de cinco sementes de plantas foi examinado contra *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* e *Candida albicans* usando o método de difusão bem. Estudos de atividade antimicrobiana revelaram alto potencial de atividade de sementes de plantas em pó de *Nigella sativa L., Cucurbita pepo*, *Sesamum radiatum*, *Trigonella foenum-graecum*, *Linum usitatissimum* com eficiência variável contra cepas microbianas testadas com concentração de 100 mg / ml, exceto *Sesamum radiatum* com pontuação não efeito. O pó de sementes de *T. foenum* e *N. sativa* apresentou a maior zona de inibição (24-20 mm) contra *K. pneumonia*, seguido por *S. aureus* (20-18 mm) e *C. albicans* (15 mm), respectivamente. O pó de cinco sementes de plantas exibiu efeitos bacteriostáticos e bactericidas com MIC's 20 e MBC 40 mg / ml contra *K. pneumonia*, enquanto MIC's 40 e MBC 60 mg / ml contra *S. aureus*. Os resultados deste estudo indicaram que os pós de sementes de plantas apresentam promissoras atividades antimicrobianas e suas potenciais aplicações em processos alimentícios. Ele poderia ser utilizado como alternativa medicinal natural em vez de substância química**.**

Palavras-chave: pó de semente de planta, antimicrobiano, intoxicação alimentar, doença.

1. Introduction

Food poisoning disease is generally known as illness or ailment induced by the consuming food polluted with bacteria, Fungi, parasites, or toxic substance. These poisonous substances have different ways to enter food chain process (from farm to table), and make them unfit for human consumption. Centers of control disease and prevention (CDC) reported that Food borne disease are causes a major problem around worldwide not especially for developing countries only, that lead to illness and death (Sapkota et al., 2012). At the United States Department of Agriculture (USDA), it is estimated that cost of foodborne illness is $15.6 billion annually. Bacteria are recorded as the most prevalent biological hazards associate with food borne diseases. The main strains of bacteria were

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isolated from food products Gram negative bacteria like; *Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa* (Pandey and Singh, 2011), and Gram-positive bacteria especially; *Bacillus cereus, clostridium species, Staphylococcus aureus* (Mostafa et al., 2018) Reduction of food poisoning diseases and their own agents are candidates by using of chemical preservatives (Shan et al., 2007). These chemical substances have efficiency role against outbreak control of food poisoning diseases, but used it abundant continuously lead to unpleasant side effect on human healthiness and accumulated in their bodies (Bialonska et al., 2010). Worldwide are focused on the use a natural food preservative to keep the health of human safe, regarded as nutritionally safe and easily degradable. Plant extracts are utilized as a natural source of antimicrobial agents and food pathogens. (Ogbulie et al., 2007; Berahou et al., 2007).

Several researchers explained the antimicrobial effect of plant extractors against food poisoning bacteria (Verma et al., 2012; Akinpelu et al., 2014), they are used as a source of natural component kept food from contamination with pathogenic bacteria and increase nutritive food value (Nasar-Abbas and Halkman, 2004). Antimicrobial activity of Petroleum ether and Ethanol Alkatan extracts were revealed significant inhibitory effects against Gram positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*). The clearest activity was seen against *K. pneumoniae* using Petroleum ether Alkatan extract at concentration 50mg/cm3, while ethanol Alkatan concentration 200mg/cm3 against *B. cereus* (Al-Bayati, 2007). Flax seeds of Linum usitatissimum (Alkatan) has bactericidal role against *P. gingivalis* with concentricity at 100µl/ml, and can be applied to treatment the periodontitis therapy (Badiger et al., 2019). Raja Narender et al., (2016) suggested that the Flax seeds powder has a bacteriostatic or bactericidal and fungicidal actions.

Al-Ameedy and Omran (2019) investigated the effect of *Nigella sativa* purified oil fractions by solvents against tested bacterial strains. Both Hexane and chloroform Fractions showed strong antimicrobial activity against *E. coli, K. pneumonia* and intestinal flora with inhibition zone (IZ) ranged 18-19± 3.5 mm (Singh et al.,2005). *Staphylococcus aureus* scored a weak effect against these both fractions with (IZ 8.6 ± 3.3 mm) (Salman et al., 2016). In addition, methicillin resistant *S. aureus* (MRSA)were sensitive to ethanolic *N. sativa* extract with concentricity 4 mg/disc, while the extract had a MIC range of 0.2–0.5 mg/ml (Hannan et al., 2008). Moreover, the ethyl acetate, water, and acetone fractions did not exhibit any effect against all bacterial strains. Black seeds powder recorded the lowest bactericidal concentration 47.5% contra *Salmonella typhi* (Utami et al., 2016). Some researchers concluded that methanolic pumpkin extract had effective inhibition active contra *B.subtilis, E. coli, S. aureus*, and *Pseudomonas*, while it was ineffective against *Aspergillus niger* and *Candida albicans* (Soni and Bali, 2019) with MIC's ranged from 20 to 60 mg/ml . Moreover, Pumpkin oil had highest antibacterial efficiency with *K. pneumoniae* and *Acinobacter baumannii*, and antifungal effect versus *C. albicans* at MIC's 8 to 16 µg/mL (Sener et al., 2006).

The antimicrobial impact of Fenugreek leaves, seeds and stem extract by (Methanol, Acetone and water extract) against *E. coli* and *S. aureus* were studied by Sharma et al. (2017) The highest inhibition region with potential effectiveness against *E. coli* and *S.aureus* was determined by methanol in the range 20 and 19 mm, followed by 16 mm by Acetone respectively, while the water extract showed without region of inhibition. Moreover, Ethanolic Fenugreek Seeds powder displayed the highest antibacterial effect contra *P. aeruginosa* and *S. aureus* with inhibition Zones (22 and 17 mm) (Al-Timimi, 2019).

Black sesame seed powder showed high antimicrobial actions against major periodontal pathogens like; *Fusobacterium nucleatum; Porphyromonas gingivalis*; and, *Prevotella intermedia* (Aditya et al., 2019). Heidari-Soureshjani et al. (2017) concluded that the minimum inhibitory concentration of sesame oil 32 mg/mL was effective against *S. aureus*. Rao et al. (2013) stated that the aqueous extract of sesame oil had an antimicrobial activity for *K. pneumoniae, E. coli, S. aureus, P. aeruginosa, and C.albicans.*

Previous studies are Focusing on the efficiency of plant extractors like; alcohol, aqueous, and oil of these plants; *Nigella sativa L., Cucurbita pepo*, *Sesamum radiatum, Trigonella foenum-graecum,* and *Linum usitatissimu* against food spoilage bacteria are studied in Arabian area. Therefore, the current research aims to evaluate the potential role of these plant seeds powder as an antimicrobial action against food poisonings diseases caused by *S. aureus, B. cereus. E. coli, K. pneumonia* and *C. albicans* in vitro.

2. Materials and Methods

2.1. Plant seeds powder

Five of plant seeds included in this research (Table 1) were purchased from local market of Taif, Saudi Arabia. The seeds were cleaned, disinfected, rinsed by distilled water then dried in shade. The dried plant seeds of every plant species were grounded into fine powder. The plant seeds powder was weighted, stored in a small bottle in dry place.

2.2. Antibacterial activity of the plant seeds powder

2.2.1. Bacterial strains

The own antimicrobial effect of every plant seed was assessed against four types of bacteria and one type of yeast that cause food borne diseases. Two strains of bacteria are Gram- positive such as; *Bacillus cereus* and *Staphylococcus aureus* (ATCC 25923) were isolated and identified by Abu-Zaid et al. (2016). Two strains of Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and as well as *Candida albicans* (ATCC 36232) (yeast). These strains were obtained from the culture collection of Microbiology laboratories department; faculty of science Cairo University, Egypt.

2.2.2. Inoculum's preparation

Each strain was sub-cultured on Mueller-Hilton agar slants at 37◦C 24 hours. The cell harvest was used for

Table 1. The ethnobotanical data of used plant seeds species.

Plant species	Family	Local name	Common name
Nigella sativa L.	Ranunculaceae	Habat albarika	Black cumin
Cucurbita pepo	Cucurbitaceae	Alqare	Pumpkin
Sesamum radiatum	Pedaliaceae	Alsamsim	Sesame
Trigonella foenum-graecum	Fabaceae.	Alhalba	Fenugreek
Linum usitatissimum	Linaceae	Alkatan	Flax(lin)

microbial activity contained viable cell count of 10^7colony forming units (CFU /ml) by MacFarland. Briefly, the antimicrobial activity determined by 0.5 MacFarland, the freshly inoculum was adjusted for each test by incubation the nutrient broth overnight at 37◦C.

2.2.3. Antibacterial activity of plant seeds powder

The Antimicrobial effect of all plant seeds powder assessed by well diffusion technique. Fifteen ml of agar media of Mueller-Hilton was poured into sterilized Petri dishes (as a basis layer) , followed by each examined organism previously as microbial suspension (one hundred ml of media per 1 ml of 10^7 CFU), then pour 15 ml of Mueller Hinton Agar (BD Difco) to attain 10^5 CFU/ml of organism (as a second layer) . Plant seed powder concentrates (100 mg/ml media) loaded into the well (8 mm in diameter) was placed on the top of Mueller-Hilton agar dishes. The dishes were kept in the refrigerator at 5 ◦C for 2 - Hours. Filter paper disc filled with 5 mg of Pencilline were used as a positive control. The antimicrobial vigor was assessed by calculating the diameter of the inhibition region around the well. The averages of three replicates were calculated.

2.2.4. Evaluation of Minimum Inhibitory Concentrations (MIC's) of the effective plant seeds powder

The minimum concentration of the antimicrobial agent which inhibits the microbial inoculum after 24 -hours of incubation period is known as MIC. Plant seeds powder with varied concentrations (10, 20, 30, 40, 50, 60,70,80,90 and 100 mg/ml) were filled their needful amount on the discs (diameter 8 mm). The discs were loaded with different concentrations of plant seeds powder on the Mueller-Hilton agar media. All plates preserved in the refrigerator Two Hours at 5 ◦C after that incubated twenty-four Hours at 37 ◦C. The inhibition regions were calculated by the diameter of clear zone round the disc then record results versus to every concentration of the plant seeds powder. The averages of three replicates were calculated.

2.2.5. Evaluation of Minimum Bactericidal Concentrations (MBC's) of the efficient plant seeds powder

The discs are taken from the two minimum concentrations of the plant seeds powder dishes showed no growth (from clear inhibition region of MIC dishes), then inoculates under aseptic conditions on sterilized plate of Tryptone soya agar (TSA). The Dishes were kept in the incubator for 24-Hours at 37 ◦C. then microbial growth was examined with the equivalent to plant seed powder concentration. The concentration of plant seeds powder did not show any bacterial growth on the freshly inoculated tryptone soy agar was recorded as MBC.

2.3. Statistical analysis

The diameter of inhibition zone data was analyzed by SAS program using one way ANOVA. The results were expressed as mean (±SD). The data at $p < .05$ were considered significant.

3. Results

3.1. Antimicrobial activity

Table 2 and Figure 1 showed the effect of plant seeds powder against microbial strains including; Gram *ve*+ bacteria (*B. cereus, S. aureus*), Gram *ve*- bacteria (*E. coli, K. pneumonia*), and *C.albicans*. *S. radiatum* seeds showed no effect against all examined strains. However, other plant seeds exhibited different inhibitory effect for both gram positive, gram-negative bacteria and yeast. *T. foenum* exhibited the most inhibitory effect against five microbial growth (*B. cereus, S. aureus, C.albicans, E. coli & K. pneumonia*) at concentration of 100 mg/ml , Followed by *N. sativa* , and *L. usitatissimum* seeds. Whereas, *Cucurbita pepo* seeds was effective against four of them (*S. aureus, K. pneumonia, c. albicanse & E. coli*). The most sensitive strain was *K. pneumonia* against seed plants followed by *S. aureus, C. albicans*, while *E. coli* and were the most resistance strains to the plant seeds respectively. The highest inhibition zone of *T. foenum* (24 mm) and *N. sativa* seed (24 mm) as comparable with the positive control, Pencilline (10 mm), against *K. pneumonia*. While, The lowest inhibition zone of C. pepo seeds powder 7 mm, 10 mm, and 12mm against *E. coli, S. aureus* and *K. pneumonia* respectively as comparable with the positive control.

3.3. Minimum Inhibitory Concentrations (MIC's) of the effect of plant seeds powder

The MIC of the most potent plant powder (*T. foenum* and *N. sativa* seed) were examined by method of well diffusion to estimate their bacteriostatic and bactericidal activates. Table 3 and Figure 2 revealed that the minimum inhibitory effect of *T. foenum* and *N. sativa L.* seeds were at 20 mg/ml with inhibition zones of 8.9 and 7.5 mm against *K. pneumonia*, while these plant seeds powder at 40 mg/ml concentration of inhibited bacterial growth of *S. aureus with* inhibition zones of 9.3 and 7.4 mm respectively.

Table 2. Antimicrobial activities of some plant seeds powder against some microbial strains of food poisoning diseases.

Plant seeds Species	Inhibition Zone (mm)				
	Gram Negative Bacteria		Gram Positive Bacteria		*yeast*
	E. coli	***K. pneumina***	***B. cereus***	***S. aureus***	***C.albicans***
Nigella sativa L.	15.3 ±0.57	20.5±0.57	13.1±0.32	18.6±0.56	15.2±0.11
Cucurbita pepo	7.0 ±0.44	12.6±0.54	0.0 ± 0.0	10.4±0.48	10.3±0.37
Sesamum radiatum	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Trigonella foenum-graecum	15. 8±0.45	24.5±0.53	13.7±0.55	20.6±0.57	15.6±0.59
Linum usitatissimum	12.4 ±0.32	15.2±0.33	10.4±0.50	13.2±0.43	13.2±0.31
Pencillin (5 mg)	0.0 ± 0.0	10.1±0.22	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Data are means of three replicates (n = 3) ± SD.

Table 3. Minimum Inhibition Concentration of the most effective plant seeds powder against *S. aureus* and *K. pneumina*.

Plant Seeds Species	Conc. mg/ml	Inhibition Zone (mm)	
		Gram-Negative Bacteria	Gram-Positive Bacteria
		K. pneumina	***S. aureus***
Trigonella foenum-graecum	10.0	0.0 ± 0.0	0.0 ± 0.0
	20.0	8.9±0.56	0.0 ± 0.0
	30.0	12.6±0.58	0.0 ± 0.0
	40.0	15.8±0.49	9.3±0.42
	50.0	17.5±0.40	12.5±0.48
	60.0	19.8±0.39	15.8±0.42
	70.0	20.2±0.45	17.2±0.50
	80.0	22.7±0.38	21.7±0.24
	100.0	25.3±0.58	22.7±0.56
Nigella sativa L.	10.0	0.0 ± 0.0	0.0 ± 0.0
	20.0	7.5 ± 0.0	0.0 ± 0.0
	30.0	8.6.0 ± 0.0	0.0 ± 0.0
	40.0	10.8±0.36	7.4.0 ± 0.43
	50.0	12.5±0.59	15.0 ± 0.67
	60.0	15.8±0.57	18.8±0.54
	70.0	18.2±0.43	20.2±0.23
	80.0	19.7±0.48	22.7±0.38
	100.0	21.7±0.24	20.7±0.35

Data are means of three replicates (n = 3) ± SD.

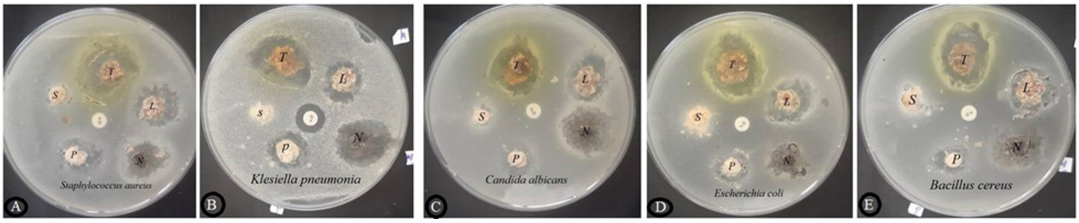

Figure 1. Growth inhibition of some food poisoning microbial strains caused by plant seeds powder. N, *Nigella sativa*; P, *Cucurbita pepo*; S, *Sesamum radiatum*; T, *Trigonella foenum-graecum; L, Linum usitatissimum* and +C, positive control.

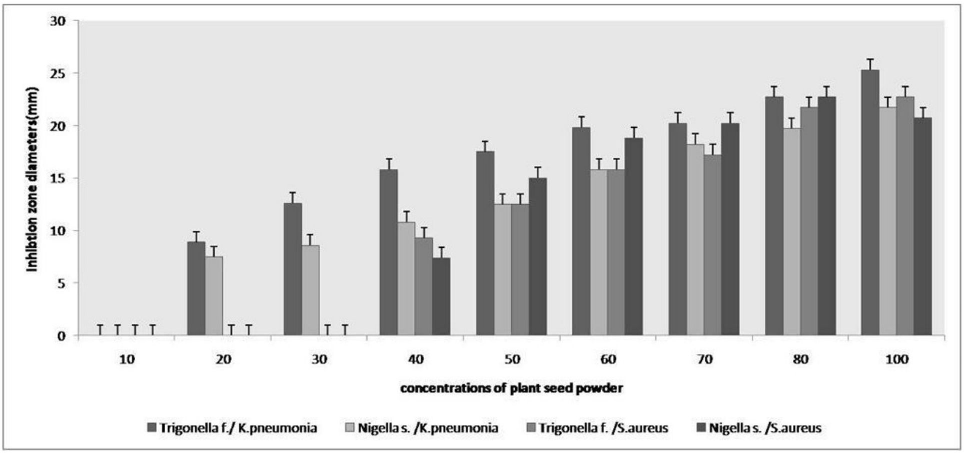

Figure 2. MIC's of the effective plant seeds powder against *S. aureus* and *K. pneumonia*, ± standard error.

3.4. Minimum Bactericidal Concentrations (MBC's) of the most effective plant seeds powder

The MBC was confirmed from the absence of bacterial growth of the examined strains-streaked form the lowest MIC's. *T. foenum* and *N. sativa* seeds exhibited bactericidal activity against *K.pneumonia* with MBC 6 mg/ml, while MBC of these plant seeds against *S. aureus* were 80 mg/ml. From these results *T. foenum* and *N. sativa* seeds were scored lowest concentration from MIC and MBC against *K.pneumonia*, followed by *S. aureus*. These plant seeds can be used to decrease or prevent food borne bacteria and food poisoning diseases. These strains of Bacteria used in this work caused a major problem in food, which were a source of food spoilage and food poisoning. MIC and MBC results of the potent plant seeds powder indicated that *T. foenum* and *N. sativa* seeds can be utilized to rule and prohibit bacteria of food borne, prevent bacterial toxins and food poisoning diseases. Whereas, *B. cereus, E. coli, S. aureus* and *K.pneumonia* the main cause of food borne disease ,because they produce toxins and other metabolites that cause human intestinal diseases. At the present data *K.pneumonia* was the most sensitive bacteria suppressing by plant seeds (*T. foenum* and *N. sativa*) and scored lowest MIC at 20 mg/ml.

4. Discussion

The difference in resistance of Gram *ve*$^+$ bacteria as *S. aureus*, *B. cereus* and Gram *ve*$^-$bacteria as *K.pneumonia , E. coli* to plant seeds powder, it could be related to differences in the cell membranes. These results are in the same line with those reported by (Kim et al., 2011). The selective barrier outer membrane Gram *ve* $^-$bacteria are hydrophilic molecules, the ion channel of this bacteria is very small to allow internalization of the plant seeds, while Gram *ve*$^+$ bacteria have a thick peptido-glycan layer that contains teichoic and lipoteichoic acid (Amato et al., 2011).

Our study revealed that Plant seeds had antimicrobial activity on the food poisoning bacteria and with varied inhibition zone. Variation in MIC of all plant seeds were used may increase according to chemical constituents and volatile nature. These results are in accordance with (Badiger et al., 2019; Soni and Bali, 2019; Sharma et al., 2017).

Zone inhibition of *T. foenum* seeds were found at (100 mg/ml) concentration against *B. cereus, S. aureus, E. coli, C. albicans and K.pneumonia* suppressing their growth with zones ranged from 13-24mm. These results were in agreement with Sharma et al. (2017) and Thomas et al. (2011). *N. sativa* scored inhibition zone range 13-20 mm and MIC from 20 to 40 mg/ml against *K.pneumonia* and S. aureus . Our results are in the same line of Sheikh et al. (2010) and (Dua et al., 2013) who revealed that the potentially effect of these plant seeds with MIC range (6.25 to 12.5 mg/ml. (Dixit et al., 2005; Halliwell, 1995) were confirmed the competency of plant seeds as antimicrobial agents due to their effective natural components to control growth of food poisoning bacteria and spoilage. These components were suggested by researchers like; (terpenoid, alkaloid, isoflavones, antioxidant and phenolic compounds), which may inhibit enzymes necessary for amino acids biosynthesis or interact with enzymes and proteins of the microbial cell membrane causing inactivation of protons in the direction outside the cell led to cell death (Burt, 2004; Gill and Holley, 2006). Badiger et al. (2019) reported that hull lignans of flaxseed have anti-microbial activity against the bacterial species. Other studies attributed the inhibitory effect of these plant extracts to their hydrophobicity characters which enable them to interact with microbial cell membrane and mitochondrial that disturb their structures and alter their permeability (Friedman et al., 2004; Tiwari et al., 2009).

5. Conclusion

The present study suggested that plant seeds powder which proved to be potentially effective can be used as natural preservatives to control food poisoning diseases and preserve food avoiding application of health hazards of chemical preservatives.

Declaration of conflict of interest

None.

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