Food Science and Nutrition Studies
ISSN 2573-1661 (Print) ISSN 2573-167X (Online)
Vol. 1, No. 1, 2017
www.scholink.org/ojs/index.php/fsns

Evaluation the Antimicrobial Activity of Artemisia and Portulaca Plant Extracts in Beef Burger

Marwa Al-Moghazy[1], M. S. Ammar[2], Mohamed M. Sief[3] & Sherif R. Mohamed[3*]

[1] Dairy Science Department, Food Industries and Nutrition Division, National Research Centre, Dokki, Giza, Egypt

[2] Food Science and Technology Department, Faculty of Agriculture, AL-Azhar University, Cairo, Egypt

[3] Food Toxicology and Contaminants Department, Food Industries and Nutrition Division, National Research Centre, Dokki, Giza, Egypt

[*] Sherif R. Mohamed, E-mail: sheriframzy4@gmail.com

Received: April 9, 2017 Accepted: April 23, 2017 Online Published: May 15, 2017

doi:10.22158/fsns.v1n1p31 URL: http://dx.doi.org/10.22158/fsns.v1n1p31

Abstract

Medicinal plants contain substances can alternate the traditional chemical preservatives which used for preserving meat products that have negative effects on consumer health. Several biological activities have been reported for Artemisia and Portulaca as antimicrobial agents, so the current study focused on using Artemisia and Portulaca extracts as antimicrobial agents in beef burger. Phytochemical of Artemisia and Portulaca extracts were analyzed, and both extracts contain alkaloids, flavonoids, phenols, trepenoids and saponin. The results show that Artemisia extract was inhibited all tested microorganisms (Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella Typhimurium, Listeria monocytogenes and E.coli O157:H7) while, Portulaca extract affect Staphylococcus aureus only. The Minimum Cidal Concentration (MCC) and Minimum Inhibitory Concentration (MIC) were carried out for testing microorganisms, since Artemisia extract was very effective against Staphylococcus aureus followed by E.coli, Pseudomonas aeruginosa, Salmonella Typhimurium and L. monocytogenes. Artemisia and Portulaca extracts were separately applied in beef burger as antimicrobials at levels 1% and 1.5%. The sensory evaluation of treated beef burger showed no significant differences between control sample and treatments containing Portulaca extract while, the addition of Artmesia extract had a detrimental effect on taste of beef burger since it causes formation of bitter taste.

Keywords

antimicrobial, Artemisia, Portulaca, plant extracts, beef burger

1. Introduction

Nowadays, new preservation techniques are being developed to extend the storage time with maintaining both the natural appearance and safety of fresh meat (Patsias et al., 2008; Zhou et al., 2010). Synthetic preservatives such as nitrite has been widely used for preserving meat products such as sausages and luncheon meats. Nitrite is efficient for inhibition of *Clostridium botulinum* (Gibson et al., 1984). Nitrite may react with secondary amines naturally present in the meat to form N-nitrosamines, which are carcinogenic (IARC, 1998). There is an association between consumption of processed meat and increase risk of different cancers (Santarelli et al., 2008; Larsson et al., 2006; Larsson & Wolk, 2012), which cause death (Rohrmann et al., 2013). Unlike synthetic compounds, natural extracts obtained from plants are rich in phenolic compounds which can enhance the overall quality of food by decreasing lipid oxidation and microbial growth (Zhang et al., 2016).

Artemisia vulgaris is used in asthma emmenagogue, anthelmintic, and stomachic (Ambasta, 1994). The essential oil of leaves exhibited significant antimicrobial activity (Laxmi & Rao, 1991). The leaves have an antibacterial activity on S*taphylococcus aureus, Bacillus typhi, Bacillus dysenteriae, Streptococci, Escherichia coli, Bacillus subtilis* and Pseudomonas.

Pharmacological studies on *Portulaca oleracea* showed its activities as antibacterial (Chan et al., 2015), hepatoprotective (Al-Sheddi et al., 2015), anti-inflammatory, analgesia (Zhou et al., 2015), antioxidant (Liu et al., 2015). So the goal of this work is the evaluation of antimicrobial effect for both *Artemisia* and *Portulaca* extracts against microorganisms in vitro and as a preservative agent in beef burger.

2. Materials and Methods

2.1 Materials

Herbs: Artemisia vulgaris and Portulaca oleracea were obtained from Egyptian herbal market, Dokki, Giza.

Microbial strains: Three Gram negative pathogen bacteria; Salmonella Typhimurium (ATCC 14028), E. coli O157:H7 and Pseudomonas aeruginosa and 2 Gram positive pathogens; Staphylococcus aureus (25923), and Listeria monocytogenes (ATCC 7644), Microbial strains are generously given by Microbiology Department, Faculty of Agriculture, Cairo University. Different microbial strains were preserved at -20^{o}C.

Ingredients of beef burger:

a. Beef meat: Frozen beef, lean meat was obtained from the local butcher shop in the day before the experiment. The meat was cold stored at 5±1 ℃ overnight.

b. Soybean flour: Soybean flour was obtained from food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

c. Other ingredients: Spices, Fresh eggs, onion and salt were obtained from the local market. While, sodium tripolyphosphate, and sodium ascorbate were obtained from the Adwic Laboratory Chemicals Co., Cairo, Egypt.

2.2 Methods

2.2.1 Technological Methods

Preparation of Artemisia vulgaris and Portulaca oleracea extract: One hundred grams of Artemisia vulgaris or Portulaca oleracea leaves were added to excessive distilled water: ethanol (2:8 v/v) and incubated at room temperature for 24 h, then the slurry was filtered through filter paper. The water extract was concentrated using a rotary evaporator under reduced pressure and the residues were dissolved in 50 ml of distilled water.

Beef burger formulation: Beef burger patties were processed by the method described by (Oroszvári et al., 2005; Ou & Mittal, 2006) according to the formula showed in Table 1:

Table 1. Beef Burger Formulation

Main ingredients (%)	Treatments				
	Control	Artemisia extract		Portulaca extract	
		1%	1.5%	1%	1.5%
Beef meat	62	62	62	62	62
Soy flour	12	12	12	12	12
Iced water	10	10	10	10	10
Fresh eggs	7	7	7	7	7
Fresh onion	7	7	7	7	7
Salt	1.5	1.5	1.5	1.5	1.5
Spices	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Additives (%)					
Sodium tripolyphosphate	0.3	0.3	0.3	0.3	0.3
Sodium ascorbate	0.03	0.03	0.03	0.03	0.03
Artemisia extract		1	1.5	1	1.5
Portulaca extract		1	1.5	1	1.5

2.2.2 Analytical Methods

DPPH radical scavenging method: The antioxidant activity of the plant extracts was evaluated using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) according to a modified method of Bandoniene et al. (2002).

Qualitative Phytochemical Analysis: phytochemical of plant extracts was carried out by standard methods described by (Brain & Turner, 1975; Evans, 1996) as follows:

Detection of Alkaloids: Extracts were dissolved separately in dilute hydrochloric acid and filtered. The filtrates were used to detect the alkaloids by using Mayer's test, since filtrates were treated with Mayer's reagent. Formation of a yellow, cream precipitate indicates the presence of alkaloids.

Detection of Saponins: About 0.5 mg of the extract was mixed by agitation with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of

saponins.

Detection of Tannins: A small quantity of extract was mixed with water and heated using water bath. Then the mixture was filtered and ferric chloride was added to the filtrate. The formation of dark green colour indicates the presence of tannins.

Detection of Flavonoids: Detection of flavonoids was carried out as follows:

-Lead acetate test: Extracts were treated with a few drops of lead acetate solution. The formation of yellow color indicates the presence of flavonoids.

-H_2SO_4 test: Extracts were treated with a few drops of H_2SO_4. Formation of orange color indicates the presence of flavonoids. Precipitate indicates that the presence of flavonoids.

Detection of Phenols: Detection of phenols was carried out as follows:

-Ferric chloride test: 10 mg extracts were treated with a few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

-Lead acetate test: 10 mg extract was treated with a few drops of lead acetate solution. Formation of a yellow color precipitate indicates the presence of phenol.

2.2.3 Quantitative Phytochemical Analysis

Estimation of Alkaloids was carried out by using the method of (Harborne, 1973) as follows: One gram of extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and the beaker was covered and allowed to stand for 4 h, then the mixture is filtered and concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added by drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH, then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids was carried out as follows: One gram of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No.1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah et al., 2009).

Estimation of Total Phenols was carried out as follows: The fat free sample was boiled with 50 ml of ether for extraction of phenolic compounds for 15 min. Five ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. This was read spectrophotometry at 505 nm.

Screening of antimicrobial activity: Screening of antimicrobial activity was performed using the agar diffusion method. The Nutrient agar plate was over layered with approximately 2 mL soft agar inoculated with 105-106 cfu/mL of overnight activated microbial cultures, then wells of 8 mm diameter were holed by cork borer, 60 µL of each tested compounds were injected in every well. Negative control was performed using sterile distilled water. Plates were incubated for 24 h at 37^{o}C. Diameters of inhibition clear zones were measured using graded ruler.

2.2.4 Microbiological Methods

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Cidal Concentrations (MCC): Determination of Minimum Cidal Concentrations (MCC) of extracts were performed using microplate methods, 100 µL of sterile nutrient broth were loaded in each well of 96 wells plate, then defferent volumes of extract were added to achieve final concentrations ranged from1 to 15% (v/v), then the inoculums of different pathogens were added to have 106 CFU/mL final concentration. After incubation for 24 h at 37^{o}C, bacterial count of each sample was determined using the drop plate method (Naghili et al., 2013). Drops of 10 µL from each well were pipetted onto surface of solidified nutrient agar, and then the plates were incubated for 24 h at 37^{o}C. Minimum inhibitory concentration defined as the concentration which shows no increase or decrease of the initial counting after 24 h of incubation, while Minimum Cidal Concentration (MCC) defined as the less concentration showed no bacterial viability.

Microbiological assessment of frozen beef burger: Microbiological assay of the different beef burger samples was carried out at zero time and after 3 months of frozen storage. Total count of bacteria was determined to evaluate the antimicrobial activity of plant extract in a real food system such as beef burger and the ability of this extract to prolong the storage period of beef burger. Ten g of each sample was added to 90 ml of sterile pepton water and mixed well for 1 min to homogenize. Decimal dilutions in sterilized pepton water were prepared and 1 ml was poured in Plate count agar (PCA, CM0325, Oxoid) to enumerate total bacterial count after incubation for 24 h at 37^{o}C.

2.2.5 Statistical Analysis

One-way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significant differences between the means were determined by Duncan's multiple range test and $p<0.05$ were regarded as significant (Sokal & Rohlf, 1995).

3. Results

Table 2 shows the qualitative phytochemical of Artemisia and Portulaca Extracts. From the obtained results it cleared that both extracts contain alkaloids, flavonoids, phenols, trepenoids, saponin while the tannins was positive in Artemisia and negative in Portulaca extract.

Table 2. Qualitative Phytochemical Analysis of Artemisia and Portulaca Extracts

Phytochemicals	Artemisia extract	Portulaca extract
Alkaloids		
Mayer s test	+	+
Flavonoids		
Lead acetate test	+	+
H_2SO_4 test	+	+
Phenols		
Ferric chloride test	+	+

Lead acetate test	+	+
Terpenoids	+	+
Saponin	+	+
Tannin	+	-

Note. (+) Present; (-) Not detected.

Table 3 shows the quantitative phytochemical of Artemisia and Portulaca Extracts. From the results it cleared that both extracts contained Alkaloids, Flavonoids, phenol and antioxidant activity at levels 4.25-5.34, 14.34-9.46, 9.47-3.85 W/w, 74%-68% respectively.

Table 3. Quantitative Phytochemical Analysis of Artemisia and Portulaca Extracts

Phytochemicals	Artemisia extract (W/w)	Portulaca extract (W/w)
Alkaloids	4.25	5.34
Flavonoids	14.34	9.46
Phenol	9.47	3.85
Antioxidant Activity	74%	68%

Table 4 shows the Artemisia and Portulaca water extract against pathogenic bacteria. The obtained results showed that Artemisia extract has a potent antimicrobial effect against all tested microorganisms, *Staph aureus, Pseudomonas aeruginosa, Salmonella Typhimurium, Listeria monocytogenes and E.coli O157:H7*. The maximum effect of the Artemisia extract as an antibacterial was against *Pseudomonas aeruginosa* and gave 28 mm Inhibition zone diameter followed by Listeria monocytogenes, *Staph aureus, Salmonella typhimurium* and *E.coli* O157:H7 since the extract could inhibit the microbial growth and the inhibition zones were 26.25, 26, 24 and 15.4 mm respectively. The Portulaca extract was also tested as an antimicrobial against the same microorganisms, but it had a potent effect only against *Staph aureus* while, did not have any effect against *Pseudomonas aeruginosa,* Salmonella Typhimurium, Listeria monocytogenes and *E.coli* O157:H7.

Table 4. Screening of Antimicrobial Activity (Inhibition Zone Diameter in mm) of Artemisia and Portulaca against Tested Pathogenic Bacteria

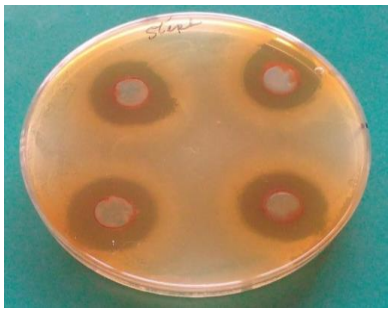
Microorganism	Artemisia extract	Portulaca extract
Staph aureus	26	20
Pseudomonas aeruginosa	28	Nil
Salmonella typhimurium	24	Nil
Listeria monocytogenes	26.25	Nil
E.coli O157:H7	15.5	Nil

Table 5 shows the Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentrations

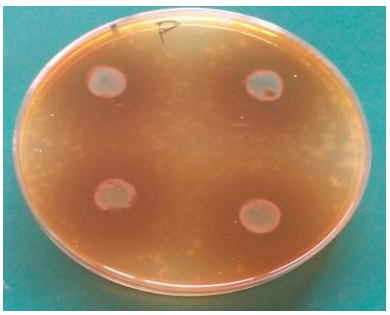
(MCC) of Artemisia extract against pathogenic bacteria (Portulaca extract was excluded because that it had not antimicrobial activity except against *Staph aureus* only). The *Minimum* Cidal *Concentration* (MCC) is the lowest *concentration* of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution *Minimum* Inhibitory *Concentration* (MIC) tests by subculturing to agar plates that do not contain the test agent. The Minimum inhibitory concentration MIC and Minimum Cidal Concentration (MCC) were carried out for testing pathogenic bacteria *are E. coli*, *Pseudomonas aeruginosa*, *Staph aureus*, *Salmonella Typhimurium*, *L.monocytogenes*. The MIC was regarded as the lowest concentration of the extracts that prevent the growth of any tested bacterial colony on the medium. From the obtained results in Table 4 the Artemisia extract was very effective on *Staph aureus* and the MIC was appeared at 5 µL/100 µL, while MIC for both *E.coli* and *Pseudomonas aeruginosa* were medium and detected at 7 µL/100 µL, moreover *Salmonella Typhimurium and L.monocytogenes* possess the lowest MIC which recorded at 9 µL/100 µL. Also the minimum cidal concentration MCC was carried out and found that the results related and agreed with the results of MIC and the MCC at 6 µL/100 µL was for *Staph aureus* followed by *E.coli*, *Pseudomonas aeruginosa and Salmonella Typhimurium*, *L.monocytogenes* at 8, 10 µL/100 µL respectively.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentrations (MCC) of Artemisia Extract against Pathogenic Bacteria

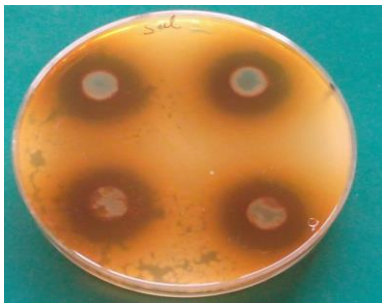
Microorganism	MIC (µL/100 µL)	MCC (µL/100 µL)
E.coli	7	8
Staph aureus	7	8
Pseudomonas aeruginosa	5	6
Salmonella typhimurium	9	10
Listeria monocytogenes	9	10



Staph aureus



pseudomonas aeruginosa



Salmonella typhimurium

Listeria monocytogenes

E.coli O157:H7

Figure 1. The Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentrations (MCC) of Artemisia Extract against Pathogenic Bacteria

Data in Table 6 show changes in the total bacterial count of beef burger during freezing, storage which was determined at zero time and after three months of frozen storage. The results show that Portulaca extract had a slight effect on beef burger microbial quality, but the rate of growth was slow which may be due to the polyphenols content of Artemisia extract which cause reducing the growth rate and maximum growth population and/or extending the lag-phase of the target microorganism (Zhou et al., 2010). The Artemisia extract possesses a potent effect as an antimicrobial agent against all tested bacteria opposite the Portulaca extract that had only effect against *Staph aureus*, but unfortunately based on the sensory evaluation of beef burger results the Artemisia extract has a detrimental effect on the taste of burger patties which impart bitter taste and were unpalatable. So, more researchers are required to remove the bitterness of Artemisia extract until we can be used it in beef burger preservation without its affecting the sensory properties.

Table 6. Microbiological Changes in Total Bacterial Count (cfu/g) Analysis of Beef Burger Samples during Frozen Storage Period

Storage time	Treatments				
	Control	Artemisia extract		Portulaca extract	
		1%	1.5%	1%	1.5%
Zero time	2.40 E+06	3.70 E+06	2.10 E+06	2.66 E+06	2.68 E+06
3 months	3.07 E+06	3.85 E+06	2.86 E+06	3.00 E+06	3.40 E+06

Table 7 shows that there is no significant differences (P≤0.05) between control and treatments containing Portulaca extract in all sensory properties of beef burger, furthermore addition of Portulaca extract with 1.5% was enhanced the taste, tenderness, juiciness and overall acceptability of beef burger. On the other hand the addition of Artmesia extract had a detrimental effect on taste of beef burger since it impart it bitter taste. Also addition of Artmesia extract was slightly lower the sensory score of beef burger for tenderness and juiciness but did not affect color and appearance.

Table 7. Effect of Addition Plant Extracts on Sensory Characteristics of Beef Burger

Sensory characteristics	Treatments				
	Control	Artemisia extract		Portulaca extract	
		1%	1.5%	1%	1.5%
Color	8^{a}	8^{a}	8^{a}	8^{a}	8^{a}
Taste	8^{a}	1^{b}	1^{b}	8^{a}	9^{a}
Flavor	8^{a}	1^{b}	1^{b}	8^{a}	8^{a}
Tenderness	8^{ab}	7^{b}	7^{b}	8^{ab}	9^{a}
Juiciness	8^{ab}	7^{b}	7^{b}	8^{ab}	9^{a}
Appearance	8^{a}	8^{a}	8^{a}	8^{a}	8^{a}
Overall acceptability	8^{a}	1^{b}	1^{b}	8^{a}	9^{a}

* Scores ranging from 0-3 = very poor, 4 = poor, 5 = fair, 6-7 = good and 8-10 = very good.

** In rows means have the same superscript are not significantly different.

4. Discussion

Our obtained phytochemical analysis of Artemisia and Portulaca extracts in agreement with (Alireza et al., 2013) who showed that tannins, saponins, alkaloids, amino acids, phenolic compounds, quinines and terpenoids are present in Artemisia extract using mass gas-chromatograph. Also, Okafor (2014) studied the water extract of aerial parts of Portulaca and showed that it contain steroids, protein, and alkaloids.

The Artemisia extract had a potent effect as an antimicrobial against all tested microorganisms while, Portulaca extract had a potent effect against *Staph aureus* only. In our respect study (Abdul & Waheeta, 2010) showed that Artemisia nilagirica extracts had a broad spectrum of antibacterial activity against phytopathogens and clinical pathogens except *S. aureus, E. faecalis and K. pneumoniae*.

Our results showed that plant extracts contain effective biological compounds such as alkaloids, flavonoids, phenols, tannins and terpenoids, which could be alternate the traditional chemicals to inhibit phytopathogenic bacteria and decrease the negative effects of synthetic drugs.

The MIC of Artemisia extract that gave a positive effect against all tested microorganisms was analyzed and the results was in agreement agreed with (Abdul & Waheeta, 2010) who studied the effects of various Artemisia extracts against bacteria and reported that hexane extract was effective

against all phytopathogens with low MIC of 32 μg/ml, while the methanol extract showed higher inhibition activity against *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella typhi*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa* (32 μg/ml), *Bacillus subtilis* (64 μg/ml) and *Shigella flaxneri* (128 μg/ml).

The MIC analyses of clinical pathogens showed an activity against Gram-positive and Gram-negative bacteria, which indicate that plant extracts contains several antimicrobials.

The phytochemical screening of plant menthol extracts showed that it contain flavonoids, terpenoids, phenols, amino acids, alkaloids and tannins, which have antimicrobial properties (Fernandez et al., 1996; Mendoza et al., 1997; Amaral et al., 1998; Cowan, 1999 ; Shaheen et al., 2003; Amarowicz et al., 2008; Chowdhury et al., 2008).

Artemisia and Portulaca extracts were incorporated into the beef burger formula as an antimicrobial agent and both extracts gave good results as antibacterial, but Artemisia extract was had superior antimicrobial activity than Portulaca extract. The sensory quality of the formulated beef burger was carried out, beef burgers incorporated with Portulaca extract were having good sensory quality, while the beef burgers containing Artemisia extract were having bad sensory quality (bitter taste), although it showed good microbiological quality, thus further researches are needed to remove the compounds which induce the bitter taste until we can use the Artemisia extract as an antimicrobial in meat products.

5. Conclusion

Finally, Beef burger can be formulated with incorporation of Portulaca extract as antimicrobial without any detrimental effect on its sensory properties, whereas more researches are needed to remove the compounds of Artemisia extract which cause the bitter taste to make it suitable for using in persevering meat products without detrimental effect on its sensory properties.

References

Abdul, R. A., & Waheeta, H. (2010). Antibacterial activity of Artemisia nilagirica leaf extracts against clinical and phytopathogenic bacteria. *Ahameethunisa and Hopper BMC Complementary and Alternative Medicine*, *10*(6).

Alireza, M., PMohammadreza, M., Khoshkholgh, P., Khosro, I., Sirus, B., & Saeed, Z. (2013). Antibacterial Activity of Essential Oils and Plant Extracts of Artemisia (Artemisia annua L.). *Vitro. Zahedan J Res Med Sci.*, *15*(6), 14-18.

Al-Sheddi, E. S., Farshori, N. N., Al-Oqail, M. M., Musarrat, J., Al-Khedhairy, A. A., & Siddiqui, M. A. (2015). Portulaca oleracea seed oil exerts cytotoxic effects on human liver cancer (HepG2) and human lung cancer (A-549) cell lines. *Asian Pac. J. Cancer Prev.*, *16*, 3383-3387.

Amaral, J. A., Ekins, A., Richards, S. R., & Knowles, R. (1998). Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. *Appl*

Environ Microbiol, *64*, 520-525.

Amarowicz, R., Dykes, G. A., & Pegg, R. B. (2008). Antibacterial activity of tannin constituents from Phaseolus vulgaris, Fagoypyrum esculentum, Corylus avellana and Juglans nigra. *Fitoterapia*, *79*, 217-219.

Ambasta, S. P. (1994). The useful plants of India. *CSIR Publication, Delhi*, 55-56.

Bandoniene, D., Murkovic, M., Pfannhauser, W., Venskutonis, P. R., & Gruzdiene, D. (2002). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. *European Food Research Technology*, *214*, 143-147.

Brain, K. R., & Turner, T. D. (1975). The practical evaluation of phytopharmaceuticals. *Wright-science technical* (1st ed., p. 144).

Chan, B. C., Han, X. Q., Lui, S. L., Wong, C. W., Wang, T. B., Cheung, D. W., … Yang, X. S.(2015). Combating against methicillin-resistant Staphylococcus aureus—Two fatty acids from Purslane (Portulaca oleracea L.) exhibit synergistic effects with erythromycin. *J. Pharm. Pharmacol.*, *67*, 107-116.

Chowdhury, N., Laskar, S., & Chandra, G. (2008). Mosquito larvicidal and antimicrobial activity of protein of Solanum. *BMC Compl Alt Med*, *8*, 62-67.

Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Clin Microbiol Rev.*, *12*, 564-582.

Evans, W. C. (1966). *Trease Evans Pharmacognosy* (14th ed., pp. 119-159). London: WB Saunders Ltd.

Fernandez, M. A., Garcia, M. D., & Saenz, M. T. (1996). Antibacterial activity of the phenolic acids fractions of Scrophularia frutescens and Scrophularia sambucifolia. *J Ethnopharmacol*, *53*, 11-14.

Gibson, A. M., Roberts, T. A., & Robinson, A. (1984). Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized cured meats. VI. Nitrite monitoring during storage of pasteurized pork slurries. *Journal of Food Technology*, *19*, 29-44.

Harbone, J. B. (1973). *Phytochemical methods* (pp. 49-188). London. Chapman and Hall, Ltd.

Him-Che, Y. (1985). *Handbook of Chinese Herbs and Formulas*. Institute of Chinese Medicine, Los Angel.

IARC. (1998). IARC monographs on the evaluation of carcinogenic risks to humans. Some NNitroso Compounds. *IARC monographs on the evaluation of carcinogenic risks to humans*, *17*.

Krishnaiah, D., Devi, T., Bono, A., & Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plants. Res.*, *3*(2), 67-72.

Larsson, S. C., & Wolk, A. (2012). Red and processed meat consumption and risk of pancreatic 290 cancer: Meta-analysis of prospective studies. *Br. J. Cancer*, 1-5.

Larsson, S. C., Bergkvist, L., & Wolk, A. (2006). Processed meat consumption, dietary nitrosamines 292 and stomach cancer risk in a cohort of Swedish women. *International Journal of Cancer*, *119*, 915-919.

Laxmi, U. S., & Rao, J. T. (1991). Antimicrobial properties of the essential oils of Artemisia pallens

and Artemisia vulgaris. *Perfumerie und Kosmetik*, *72*(8), 510-511.

Liu, X. F., Zheng, C. G., Shi, H. G., Tang, G. S., Wang, W. Y., Zhou, J., & Dong, L. W. (2015). Ethanol extract from Portulaca oleracea L. attenuated acetaminophen-induced mice liver injury. *Am. J. Transl. Res.*, *7*, 309-318.

Mendoza, L., Wilkens, M., & Urzua, A. (1997). Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean Pseudognaphalium (Asteraceae). *J. Ethnopharmacol*, *58*, 85-88.

Okafor, A., & Ezejindu, N. (2014). *Phytochemical Studies on Portulacaoleracea L. Plant*, *3*(1), 132-169.

Oroszvári, B. K., Sjoholm, I., & Tornberg, E. (2005). The mechanisms controlling heat and mass transfer on frying beef burgers. The influence of the composition and comminution of meat raw material. *Journal of Food Engineering*, *67*, 499-506.

Ou, D., & Mittal, G. S. (2006). Double-sided pan-frying of unfrozen/frozen hamburgers for microbial safety using modelling and simulation. *Food Research International*, *39*, 133-144.

Patsias, A., Badeka, A. V., Savvaidis, I. N., & Kontominas, M. G. (2008). Combined effect of freeze chilling and MAP on quality parameters of raw chicken fillets. *Food Microbiol*, *25*, 75-581.

Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H. B., Jakobsen, M. U., Egeberg, R., & Tjønneland, A. (2013). Meat consumption and mortality-results from the European Prospective Investigation into Cancer and 327 Nutrition. *BMC Medicine*, *11*.

Santarelli, R. L., Pierre, F., & Corpet, D. E. (2008). Processed Meat and Colorectal Cancer: A Review of Epidemiologic and Experimental Evidence. *Nutrition and cancer*, *60*(2), 131-144.

Shaheen, F., Khan, R. A., Soobia, A., Khan, S. A., Saima, T., & Aqeel, A. (2003). New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. pendula. *Planta medica*, *69*, 350-355.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research* (3rd ed., p. 887). W.H. Freeman and Co., New York, USA.

Wright, C. W. (2002). *Artemisia*. Taylor & Francis. New York.

Zhang, H., Wu, J., & Guo, X. (2016). Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Science and Human Wellness*, *5*, 39-48.

Zhou, G. H., Xu, X. L., & Liu, Y. (2010). Preservation technologies for fresh meat. *Meat Sci.*, *86*, 119-128.

Zhou, Y. X., Xin, H. L., Rahman, K., Wang, S. J., Peng, C., & Zhang, H. (2015). *Portulaca oleracea L.*: A review of phytochemistry and pharmacological effects. *Biomed. Res. Int*.