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Characterising and optimising antioxidant and antimicrobial properties of clove extracts against food-borne pathogenic bacteria

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Abstract

Phenolic compounds present in clove extract have been reported to possess strong inhibitory effect against a vast range of microorganisms. The present work was aimed to optimise the antioxidant and antimicrobial potentials of clove extract against major food-borne pathogenic bacteria (*Salmonella Typhimurium*, *Escherichia coli*, *Listeria monocytogenes*). Results revealed that clove extract obtained from *n*-hexane extraction had higher extraction yield (48.84%), antioxidant potential (TPC = 54.05 mg/g of GAE, TFC = 15.54 mg/g, DPPH = 0.29 mg/mL, FRAP = 0.69 mg/mL) and antimicrobial activity as compared to the extracts obtained from ethanol, petroleum ether and steam distillation. Minimum inhibitory concentration of clove extract (0.25%) was more effective against *L. monocytogenes* (ZI = 11.17 mm) in comparison with *S. Typhimurium* (ZI = 8.8 mm) and *E. coli* (ZI = 9.27 mm). Additionally, clove extract was also effective in disrupting cell membrane integrity and affecting membrane permeability of the assessed bacteria. It was also found that *L. monocytogenes* was more susceptible to clove phytochemicals as compared to the other bacteria. Hence, clove extract has strong potential to be used as a natural antioxidant and antimicrobial agent in food industry.

Introduction

Products obtained from plants have been extensively used for their therapeutic applications since ancient times, and the present demand for these phytogenic products has increased. Recently, studies have been focused to extract the useful compounds from plants for applications in pharmaceutical, flavouring and food industries. Several plants have been investigated for their antioxidant and antimicrobial effects due to the presence of various functional ingredients in their essential oils. Similarly, essential oil obtained from clove buds also possesses several functional properties i.e., antibacterial, antifungal, antioxidant, antitumor, anti-inflammatory, insecticidal and flavour-imparting characteristics. Clove oil has several active ingredients that describe its functionality. It primarily contains eugenol (48 - 89%), β-caryophyllene (5 - 22%) and eugenyl acetate (0.4 - 22%). Additionally, it also possesses small quantity of α-humulene. Percentages of these functional components depend upon several factors such as variety of clove, part of plant, type of soil, plant genetics, climatic conditions and extraction methods (Khalil *et al*., 2017).

Several methods have been used to extract clove oil. The most popular methods include steam distillation, solvent extraction (Guan *et al*., 2007), hydro-distillation (Jeyaratnam *et al*., 2016), microwave-assisted extraction (González-Rivera *et al*., 2016), supercritical carbon dioxide extraction (Chatterjee and Bhattacharjee, 2013) and ultrasound-assisted extraction (Alexandru *et al*., 2013). Each method has its own advantages and disadvantages, and quantity of extracted functional ingredients depends upon the type of method used.

Essential oil obtained from clove represents considerable antioxidant properties. Antioxidant potential of clove essential oil can be explained as formation of complexes with reduced metals which inhibits peroxidation by eliminating free radicals and formation of iron-oxygen chelate complex (Ito *et al*., 2005). In a trial, Gülçin (2001) revealed antioxidant properties of clove extracts and reported 96.7% inhibition of linoleic acid emulsion peroxidation at 15 µg/mL comparable to butylated hydroxytoulene

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(BHT). In another study, Gülçin *et al*. (2004) demonstrated 93.3% and 97.9% inhibition of linoleic acid oxidation at 20 and 60 µg/mL, respectively.

Additionally, studies have revealed that clove extracts possess significant antibacterial potential against several bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The active compounds in clove extract, particularly eugenol, damage bacterial membranes and cause leakage of intracellular materials (Oyedemi *et al*., 2009). Clove extract also has inhibitory effect on the biofilms, and it is evident from studies that clove extract can reduce the formation of biofilms up to 99% (Raja *et al*., 2015). In another investigation, El-Maati *et al*. (2016) studied phenolic extracts of clove for their antioxidant and antimicrobial properties, and concluded that clove extract can be used in food and pharmaceutical products as natural antimicrobial or antioxidant agent.

Above mentioned discussion strongly supports the antioxidant and antimicrobial potential of clove extracts. Studies have revealed the effect of clove oil/extract on bacterial reduction, but no studies have been conducted on exploring the effect of applying clove extract on bacterial cell integrity and membrane permeability. So, the present work aimed to elucidate the effect of clove extracts on cell membrane permeability and integrity against different food-borne pathogenic bacteria; *Salmonella Typhimurium*, *Escherichia coli* and *Listeria monocytogenes*. Additionally, the present work also evaluated the antioxidant potential of clove extract obtained from different extraction techniques.

Materials and methods

The present work was conducted in Meat Science and Technology Laboratory (MSTL) and Food Microbiology and Biotechnology Laboratory (FMBL), National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan. In the present work, locally cultivated variety of cloves was exposed to characterisation, antioxidant potential estimation and antimicrobial assay.

Materials and chemicals

Clove buds were purchased from the local market in Faisalabad, Pakistan. All the other reagents for extraction (ethanol, *n*-hexane, petroleum ether), compositional analysis, antioxidant assay and microbial analysis were purchased from Merck (Germany) and Sigma-Aldrich (USA). The other materials were purchased from reputed scientific store of Faisalabad, Pakistan. Bacterial strains (*S. Typhimurium*, *E. coli* and *L. monocytogenes*) were provided by Institute of Food Microbiology, University of Agriculture Faisalabad.

Experimental design

The experiments for characterisation, extraction, antioxidant assay and antimicrobial analyses were performed under Controlled Randomized Design (CRD) while experiments related to bacterial cell integrity and membrane permeability were conducted under CRD using factorial arrangement.

Sample preparation and characterisation of clove buds

For the characterisation of clove, clove buds were firstly ground and then clove powder was subjected to proximate analysis.

Moisture contents

The moisture contents of clove powder were measured by drying the samples in conventional forced air laboratory oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105 ± 2°C until constant weight was achieved (AACC, 2000).

Crude protein

The percentage of crude proteins was estimated through Kjeltech Apparatus (AACC, 2000). Briefly, 1.5 g sample was taken and mixed with 5 g digestion mixture (K_2SO_4, $CuSO_4$ and $FeSO_4$) and 30 mL H_2SO_4 in digestion flask. The mixture was then subjected to mild heating (40°C) for 10 min and then temperature was raised to 60°C until the completion of digestion process i.e., turning of mixture to greenish colour. After cooling, the volume was made up to 250 mL by adding distilled water. Then, the sample was subjected to neutralisation process in distillation assembly by mixing 10 mL sample with 10 mL NaOH solution (40%) in distillation tube against boric acid solution (4%) with methyl red (indicator). Following distillation, titration was performed against 0.1 N H_2SO_4 and the volume of acid was noted.

Crude fat

Crude fat was determined by using Soxhlet Extraction System (AACC, 2000). Briefly, 2 g sample (oven dried) was taken in thimble and crude fat was extracted by using *n*-hexane after completing five siphon cycles. Next, *n*-hexane was evaporated using rotary evaporator and crude fat contents were estimated based on differences in the weights of sample.

Crude fibre

Crude fibre contents of powdered clove samples were determined by digestion of sample (fat free) with 1.25% H_2SO_4 (30 min) and then with 1.25% NaOH solution using Labconco Fibertech apparatus (AACC, 2000).

Ash

Determination of ash in clove powder was performed by charring the samples followed by placement in the muffle furnace at 550°C until the colour changed to greyish white (AACC, 2000).

Nitrogen free extract

The nitrogen free extract was calculated through subtraction method using Equation 1:

NFE% = 100 – (Moisture + Crude protein + Crude fat + Crude fibre + Ash) (Eq. 1)

Extraction of clove oil

Extraction of clove oil was done through Soxhlet extraction technique and steam distillation as described by Quan *et al*. (2004) and Guan *et al*. (2007), respectively. For solvent extraction, 30 g sample was transferred to filter paper extraction thimble and placed into the reflux flask (500 mL) followed by extraction with 250 mL organic solvents (ethanol, *n*-hexane, petroleum ether) in Soxhlet apparatus. After the completion of extraction process (3 - 4 h), the obtained extracts were concentrated on rotary evaporator at 50°C. For steam distillation, 100 g ground sample was taken in 500 mL distillation flask and subjected to steam distillation for 6 - 8 h followed by dehydration and refrigeration (4 ± 0.5°C) until further analysis.

Antioxidant activity of clove extracts

Total phenolic contents (TPC)

The total phenolic contents of clove extracts were determined by following the method described by Jang *et al*. (2008). Briefly, 125 µL clove extract was mixed with 500 µL distilled water and 125 µL Folin-Ciocalteu reagent. The resultant mixture was kept at room temperature for 5 min and then combined with 1.25 mL Na2CO3 solution (7%). Then, distilled water was added to the solution to make the total volume 3 mL. Afterwards, the mixture was kept for 90 min before taking the absorbance at 765 nm by UV-Visible Spectro-photometer. Total phenolic contents were determined and expressed as mg Gallic acid equivalent (GAE/g).

Total flavonoid contents

The total flavonoid contents were determined by aluminium chloride colorimetric method (Chang *et al*., 2002). Briefly, 1 mL methanol was added to 50 µL extract followed by the addition of distilled water (4 mL). The solution was then allowed to incubate for 5 min and 0.3 mL $NaNO_2$ (5%) and 0.3 mL $AlCl_3$ (10%) were then added. After that, 6 min incubation period was given and 2 mL NaOH solution (1 M) was added. Finally, volume of the solution was made up to 10 mL using distilled water and absorbance was taken at 510 nm in UV/vis Spectro-photometer.

Reducing ability

The ferric reducing antioxidant power (FRAP) was estimated by following the method of Benzie and Strain (1999). Briefly, sample (200 µL) was mixed with equal quantities (500 µL each) of sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%). The solution was incubated for 20 min at 50°C and centrifuged at 2,200 g for 10 min after adding 2.5 mL 10% trichloroacetic acid (TCA). Afterwards, 500 µL supernatant was taken and mixed with distilled water (500 µL) and 0.1% solution of ferric chloride (100 µL). Finally, the absorbance was measured at 700 nm using UV/vis spectrophotometer.

DPPH activity

The antioxidant capacity of resultant clove powder extracts was determined as free radical scavenging potential through DPPH (2,2 diphenyl-1-picrylhydrazyl) assay (Jung *et al*., 2010). Briefly, 4 mL extract was taken in cuvette and 1 mL DPPH solution was added to the sample. The solution was then allowed to incubate at 25°C for 30 min and absorbance was taken at 520 nm through UV-Vis Spectro-photometer. Equation 2 was used to calculate percent inhibition:

Reduction in absorbance (%) = [{AB(s) – AB(e)} / AB(s)] × 100 (Eq. 2)

where AB(s) = absorbance of blank sample (t = 0 min), AB(e) = absorbance of tested extract solution (t = 30 min)

Antimicrobial activity of clove extracts

Zones of inhibition (ZI) for clove extracts against *S. Typhimurium*, *E. coli* and *L. monocytogenes* were measured by disc diffusion method (Rahman *et al*., 2017) using clove extracts obtained from different extraction methods. Micro-wells were filled with 90 µL peptone water. After that, 10 µL of each extract was

added in the wells followed by dipping of sterilised discs having diameter of 6 mm for 1 h. Commercial antibiotics (10 µL) against *S. Typhimurium* (nalidixic acid), *E. coli* (nalidixic acid) and *L. monocytogenes* (chloramphenicol) were also added in some wells and served as positive controls. Bacterial cultures were inoculated on their respective growth media (XLT4 for *S. Typhimurium*, MacConkey Sorbitol medium for *E. coli*, MOX for *L. monocytogenes*) and discs were placed on the plates followed by incubation at 37°C for 24 h. After prescribed incubation time, the zones of inhibition were measured for each pathogen.

Selection of best treatment

Based on antioxidant activity and antimicrobial susceptibility tests, clove extract obtained from *n*-hexane was chosen for further analysis on antimicrobial activity and its effect on cell membrane integrity and membrane permeability against pathogenic bacteria.

Minimum inhibitory concentrations and zones of inhibition

Minimum inhibitory concentration (MIC) of clove extract obtained from *n*-hexane was determined by using the Kirby Bauer Method (Bauer *et al*., 1966). Firstly, stock solution of clove extract was prepared. Then, 50 µL distilled water was added to each well of micro-titration plate with the help of micro-dispenser. Afterward, 50 µL solution was added to the first well. Then 50 µL dilution was shifted to the next well and so on. Then 2 filter paper discs having 6 mm diameter were soaked in each well. Bacterial suspensions were taken and inoculated as previously mentioned, and MICs were determined after incubating the cultures at 37°C for 24 h. Similarly, the zones of inhibition were determined at different concentrations as previously mentioned.

Cell membrane integrity

Bacterial cell membrane integrity of clove extract against *S. Typhimurium*, *E. coli* and *L. monocytogenes* was determined by following the protocol of Chen and Cooper (2002). After harvesting, bacterial cells were washed and resuspended in sterilised NaCl solution (0.9%) followed by addition of 2 mL clove extract in each sample except control. Afterwards, 2 mL sample was taken from each tube at an interval of 1 h (up to 14 h), filtered with syringe filters (0.2 µm) and absorbances were taken at 260 nm using UV-Vis Spectrophotometer.

Membrane permeability

Membrane permeability was determined based on the release of cytoplasmic β-galactosidase activity from bacterial cells by using ONPG (O-nitrophenyl-β-D-galactoside) substrate. Briefly, bacterial cells were harvested, washed and resuspended in sterilised NaCl solution (0.9%). The optical densities (OD) were adjusted to 1.2 by taking absorbances at 420 nm using UV-Vis Spectro-photometer. Next, 1.6 mL bacterial suspension was mixed with 1.6 mL clove extract and 150 µL ONPG (30 mM). Finally, the absorbances were taken at 420 nm after every 10 min up to 120 min (Liu *et al*., 2015).

Statistical analysis

The obtained data were statistically analysed using Statistics 8.1 software. Analysis of variance (ANOVA) was performed to measure the level of significance. All pair-wise comparison was made through Tukey's Test (Montgomery, 2008).

Results and discussion

Compositional analysis of clove

The results of compositional analysis of clove powder are presented in Table 1. The mean values of moisture, crude protein, crude fat, crude fibre, ash and nitrogen-free extract were 10.36%, 7.62%, 16.67%, 3.14%, 3.57% and 58.64%, respectively.

Table 1. Average composition of clove powder.

Parameter	Average value (%)
Moisture	10.36
Crude protein	7.62
Crude fat	16.67
Crude fibre	3.14
Ash	3.57
Nitrogen-free extract	58.64

Extraction optimisation of clove essential oil

Extraction yield

Different solvent systems; ethanol extraction system (EES), *n*-hexane extraction system (HES), petroleum ether extraction system (PES) and steam distillation extraction system (SES) were employed for the extraction of clove essential oil, and the extraction yield of each system are shown in Table 2. The maximum extraction yield was obtained by HES (48.84%) followed by PES (43.86%), EES (41.47%) and SES (11.72%).

Solvent extraction is a preferable method to extract essential oils from plant materials due to its high extraction efficiency and less energy consumption. Hexane has maintained the dominant position

amongst all the solvents for many years. Hexane has high ability to extract oil due to the presence of several isomers. It is preferred for solvent extraction due to its high volatility and low sensible heat (335 kJ/kg). Moreover, it can be easily removed from oil or extract with low use of energy. Additionally, the azeotrope (95% hexane and 5% water) of hexane is quite efficient for the removal of contents from solids at slightly lower boiling temperature (61.6°C) by direct use of steam. Furthermore, hexane also has good dissolving properties with oils which is beneficial when washing off extracts from solids or fibrous materials (NFPA, 2009).

Antioxidant activity of clove extracts

Different phenolic compounds present in plants possess strong therapeutic properties against several life-style related ailments due to their antioxidant potential. The total phenolic and flavonoid contents, FRAP and DPPH activities of clove extracts obtained from different extraction systems are shown in Table 2. The TPCs of clove extracts ranged from 23.62 to 54.05 mg/g GAE with HES exhibiting the highest value and SES showing the least. The statistical analyses revealed that extraction system had significant ($p < 0.05$) effect on TPCs of clove extract. The differences in phenolic contents could be due to varied affinity of clove polyphenols towards different solvents. Similarly, the TFC of clove extracts were also significantly different. Maximum TFCs were recorded in HES (15.54 mg/g) followed by PES (14.63 mg/g), EES (12.46 mg/g) and SES (9.22 mg/g).

Table 2 also shows the results of FRAP and DPPH analyses of clove extracts. It was revealed that clove extract of HES showed highest DPPH activity (0.29 mg/mL) followed by PES (0.24 mg/mL), EES (0.21 mg/mL) and SES (0.15 mg/mL). Similar trend was also observed for FRAP.

Table 2. Extraction yield and antioxidant activity of clove extracts obtained from different extraction processes.

Treatments	Extraction Yield (%)	TPC (mg GAE/g)	TFC (mg quercetin /g)	FRAP (mg/ mL)	DPPH (mg/ mL)
S1	41.47^{c}	48.41^{c}	12.46^{b}	0.59^{b}	0.21^{c}
S2	48.84^{a}	54.05^{a}	15.54^{a}	0.69^{a}	0.29^{a}
S3	43.86^{b}	50.88^{b}	14.63^{a}	0.63^{b}	0.24^{b}
S4	11.72^{d}	23.62^{d}	9.22^{c}	0.32^{c}	0.15^{d}

Different superscript letters in each column indicate significant difference ($p < 0.05$). S1 = ethanol (95%); S2 = n-hexane (95%); S3 = petroleum ether (95%); S4 = steam distillation.

Several reports have demonstrated that plants contain a variety of phytochemicals such as polyphenols which are effective in scavenging free radicals (Khalil *et al*., 2017). Clove extract principally contains eugenol and iso-eugenol which are majorly responsible for its antioxidant potential. These phytochemicals form complexes with reduced metals and impose inhibitory effect on lipid oxidation by eliminating free radicals and forming iron-oxygen complexes (Ito *et al*., 2005).

Ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions are strong pro-oxidants, and promote the generation of reactive oxygen species (ROS). Similarly, free radical chain reactions also promote lipid peroxidation. Minimising the generation of ferrous ions and scavenging of free radicals delay the process of lipid oxidation in food and biological systems. Chelating agents disrupt the complex formation which results in the decrease in red colour. This discoloration is observed by spectrophotometer and chelating capacity of an antioxidant is based on the extent of the discoloration. Lower absorbance usually indicates high metal chelating activity of antioxidants. In the present work, the chelating ability of clove extract was described by inhibiting the generation of ferrous and ferrozine complex by capturing ferrous ions before ferrozine. In DPPH assay, antioxidants generally cause reduction of stable violet DPPH radicals followed by their conversion to a yellowish compound i.e., diphenyl-picrylhydrazine. The hydrogen-donating antioxidant reduces alcoholic DPPH and converts it into non-radical form DPPH-H (Gülçin *et al*., 2012). Findings of the present work strongly support the power of clove extract to reduce radical form of DPPH into non-radical form.

Antimicrobial activity of clove extracts

The antimicrobial activity of clove extracts obtained through different extraction systems (EES, HES, PES, SES) against food-borne pathogenic bacteria is demonstrated in Table 3. The highest zones of inhibition were shown by HES; 24.27 mm for *S. Typhimurium*, 25.8 mm for *E. coli* and 29.67 mm for *L. monocytogenes*. Results also suggested that *L. monocytogenes* was more susceptible to antimicrobial clove extract as compared to other pathogens. Other prepared extracts of clove also showed considerable antimicrobial potential, but their zones of inhibitions were smaller as compared to HES. Antimicrobial activity of clove extracts was compared with commercial antibiotics specific for each bacterial strain i.e., nalidixic acid was used for *S. Typhimurium* (31.57 mm) and *E. coli* (31.57 mm) whereas chloramphenicol was used for *L. monocytogenes* (38.8 mm).

Table 3. Antimicrobial activity of clove extracts against Salmonella *Typhimurium*, Escherichia coli and Listeria monocytogenes.

Treatment	Inhibition Zone (mm)		
	Salmonella Typhimurium	*Escherichia coli*	*Listeria monocytogenes*
T0	31.57[a]	31.57[a]	33.8[a]
T1	19.33[d]	20.9[d]	26.6[d]
T2	24.27[b]	25.8[b]	29.67[b]
T3	22.33[c]	22.37[c]	27.53[c]
T4	14.33[e]	15.77[e]	19.73[e]

Different superscript letters in each column indicate significant difference ($p < 0.05$). T0 = control; T1 = ethanol (95%); T2 = n-hexane (95%); T3 = petroleum ether (95%); T4 = steam distillation. For T0, nalidixic acid was used for *S. Typhimurium* and *E. coli* whereas chloramphenicol was used for *L. monocytogenes*.

The principal polyphenol present in clove extract (eugenol) possesses strong antimicrobial potential against a wide range of Gram-positive and Gram-negative bacteria. Eugenol induces bacterial cell lysis by damaging their cell wall and plasma membrane resulting in the leakage of intracellular fluids along with protein and lipid-based contents (Oyedemi *et al*., 2009).

Minimum inhibitory concentration

Based on the data obtained by extraction efficiency, antioxidant activity and antimicrobial potential, clove extract obtained by hexane-based extraction system was further assessed for its inhibitory effect. The MIC and zones of inhibition of clove extract are depicted in Table 4. Findings showed that the zones of inhibitions increased with increasing concentration of clove extract solution. No zones of inhibition were observed below 0.25% solution therefore this concentration was declared as MIC of clove solution against selected food-borne pathogens. The maximum concentration i.e., 1.5% showed highest diameters of inhibition for *S. Typhimurium* (13.73 mm), *E. coli* (16.63 mm) and *L. monocytogenes* (19.2 mm). Results of the present work undoubtedly described that clove extract has strong efficiency to inhibit the growth of pathogenic microorganisms.

Table 4. Minimum inhibitory concentrations and inhibition zones of clove extract using n-hexane as solvent against Salmonella *Typhimurium*, Escherichia coli and Listeria monocytogenes.

Treatment	Inhibition Zone (mm)		
	Salmonella Typhimurium	*Salmonella Typhimurium*	*Salmonella Typhimurium*
C0	31.3[a]	31.03[a]	34.37[a]
C1	8.8[d]	9.27[e]	11.17[e]
C2	9.87[d]	11.6[d]	13.77[d]
C3	11.53[c]	14.53[c]	17.37[c]
C4	13.73[b]	16.63[b]	19.2[b]

Different superscript letters in each column indicate significant difference ($p < 0.05$). C0 = control; C1 = 95% n-hexane extract (0.25% solution); C2 = 95% *n*-hexane extract (0.50% solution); C3 = 95% n-hexane extract (1.00% solution); C4 = 95% n-hexane extract (1.50% solution). For C0, nalidixic acid was used for *S. Typhimurium* and *E. coli* whereas chloramphenicol was used for *L. monocytogenes.*

Integrity of bacterial plasma membranes

Results illustrated in Figure 1a show that amount of 260 nm absorbing materials released from *S. Typhimurium* and *E. coli* suspensions showed quite similar patterns whereas materials released from suspension of *L. monocytogenes* showed different pattern. The OD-260 values of all bacterial suspensions increased up to 10 h which indicated good inactivation kinetics of clove extract while these values tended to decrease after 10 h.

The release of cytoplasmic components from bacterial cells can only be monitored if plasma membrane of cells is compromised. When bacterial membranes interact with antimicrobial agents, cellular materials such as phosphate and potassium ions, DNA, RNA and other protein and lipid-based compounds start coming out of the bacterial cells. These intracellular constituents can be detected through UV-Vis spectrophotometer. The values of OD taken by spectrophotometer indicate the damage of bacterial cell membrane (Franklin and Snow, 1981). Inferences of the present work show the role of clove extract in the quick release of OD-260 materials. However, the decrease in the amount of releasing cellular constituents after a certain period (10 h) is mainly due to precipitation, adsorption of releasing components to precipitates and denaturation of RNA or DNA. It was also observed that the extent of leakage of nucleotides was different in *L monocytogenes* as compared to *S. Typhimurium* and *E. coli*. This variation in nucleotide leakage was mainly attributed to the difference in cell membrane structure of Gram-positive and Gram-negative bacteria (Denyer, 1995).

Bacterial membrane permeability

The potential of clove extract to permeate the membranes of *S. Typhimurium*, *E. coli* and *L. monocytogenes* was determined as a function of releasing cytoplasmic β-galactosidase. After treating bacterial suspensions with clove extracts, a lag time of 10 min was given and after that a progressive release of β-galactosidase was seen from bacterial cells up to 100 min. However, the effect of clove extract was more prominent on *L. monocytogenes* as compared to *S. Typhimurium* and *E. coli*. However, a non-significant and steady release of β-galactosidase

Figure 1a

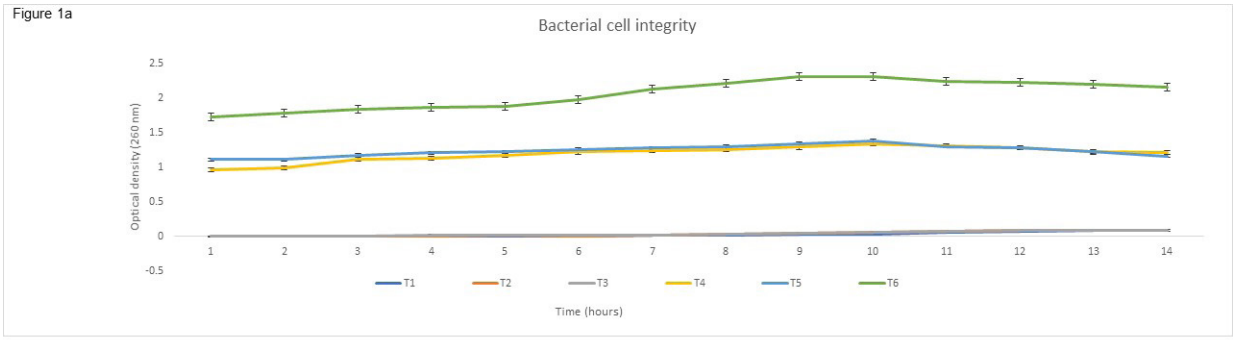

Figure 1b

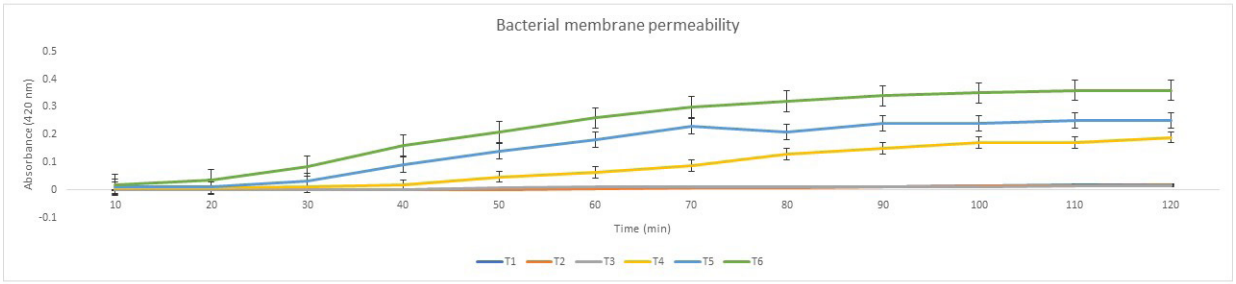

Figure 1: (a) Release of 260 nm absorbing materials and (b) Release of cytoplasmic β-galactosidase from cells of Salmonella *Typhimurium*, Escherichia coli and Listeria monocytogenes with treatment of clove extract.

was observed in control bacterial suspensions (Figure 1b). The results also revealed that the release of β-galactosidase from bacterial membranes was time-dependent.

Conclusion

Outcomes of current investigation provide sufficient evidence that clove extracts obtained from various extraction methods possessed significantly different antioxidant and antimicrobial capability based on the affinity of phytogenic complexes enclosed in the matrix of clove extracts. Total phenolic contents, flavonoids, DPPH activity and FRAP ability were reported highest in clove extract of *n*-hexane extraction system as compared to the extracts obtained from other extraction systems. Similarly, same extract showed highest antimicrobial properties against experimental pathogenic bacteria. Additionally, the results of MIC, cell membrane integrity and membrane permeability demonstrated that *L. monocytogenes* was more susceptible to clove extract as compared to *S. Typhimurium* and *E. coli*. Conclusively, the study strongly illuminated the antioxidant and antimicrobial prospective and potential application of clove extract as natural preservative. Moreover, future studies are in progress and mainly focused on the use of clove extracts as preservative in different food products based on the findings of current investigation.

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