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Cinnamon Oil as a Antimicrobial Agent to Reduce *E.coli* Contamination in Sprouts and its Effect on Quality Parameters

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Traditionally the people of India have a long-standing practice of using wide variety of herbal products in treatment of diseases or as preservatives in foods. Spices are indispensable components of Indian cuisines since ancient times. Spices are considered as rich source of bio-active antimicrobial compounds. This study was undertaken to determine the in vitro antimicrobial activity of commercial essential oil of cinnamon (spice) and its main component for potential application in sprouts for reduction of microbial contamination. The antibacterial effect against Escherichia coli was tested using paper disk diffusion method, followed by determinationof minimum inhibitory (MIC) and bactericidal (MBC) concentrations. Cinnamon essential oil exhibits antimicrobial activity against tested bacteria. The essential oil of cinnamon showed strong antimicrobial activity with MIC e" 1000ìl/ML andMBC e"4000ìl/ML.The resultssuggest that the activity of the essential oilof cinnamon can be attributed to the existence mostly of cinnamaldehyde which appear to possess similar activities against thetested bacteria. This material could be served as an important natural alternativeto prevent bacterial growth in food products.

Keywords: Spices; Antimicrobial; Bactericidal, essential oil, food safety.

Pathogenic and food spoilage bacteria have been considered as the primary causes of food-borne diseases and food quality deterioration in both developed and developing countries. In order to assure the food safety and to extend the shelf life of food products, additions of chemical preservative agents into food products or decontamination treatments via physical, chemical or biological process or their combinations have been widely applied in food industries [1]. Essential oils (EOs) can be extracted from various aromatic plants including herbs and spices as they are synthesized by these plants[2]. Spices and their EOs have beenused as natural preservatives, to produce wholesome food products, for extension of shelf-life and toreduce pathogenic bacteria [3]. Cinnamon belongs to the Lauraceae family and the genus of *Cinnamomum* which comprises of about 250 species. Cinnamon is also a traditional herbal medicine that is widely distributed in China, India and Australia[4]. It has been applied in food, seasonings, cosmetics and medical industries because of its antimicrobial, antioxidant and anti-carcinogenic activities. The antimicrobial activity of cinnamon EO and its major composition had been previously evaluated .Our study aimed to investigate the antibacterial effect of cinnamon EO by incorporating in the sprouts to provide safety to consumer and better understanding on the use

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of natural antibacterial agents[5].Sprouts present a unique food safety challenge compared to other fresh produce, as the sprouting process provides optimal conditions for the growth and proliferation of pathogenic bacteria. The sprout industry, regulatory agencies, and the academic community have been collaborating to improve the microbiological safety of raw sprouts, including the implementation of Good Manufacturing Practices (GMP), establishing guidelines for safe sprout production, and chemical disinfection of seed prior to sprouting. However, guidelines and best practices are only as good as their implementation [6]. The consumption of raw sprouts is considered high-risk, especially for young, elderly and immuno-compromised persons. By considering this all outbreaks we can conclude that there are lots of helpful ingredients and chemical which will reduce the microbial contamination. Addition of natural antibacterial agent to sprouts will help to eliminate the contamination to acceptable level. To reduce such risk at beginning level is difficult task, and to maintain the physical appearance and organoleptic properties also, so use of natural antibacterial agent will help to reduce contamination and will not deteriorate its physical appearance and organoleptic findings also[7].

MATERIALS AND METHODS

Green gram sprouts

Green gram seeds were purchased from local super market, Potheri, Chennai, India **Bacteria and Culture Conditions**

E.coli used in this study was isolated from different water samples.

Essential oil

Cinnamon oil was purchased from local shop in Chennai, India

Antibacterial assay

The antibacterial activity of Cinnamonaldehyde was evaluated by disc diffusion method. The exponential phase cultures of *E.coli* were adjusted to the concentration of 1.02×10^8 CFU/ml and were swabbed on Mueller Hinton Agar(MHA) plates. Sterile paper discs (6mm diameter) were loaded with 20ìl of different of Cinnamonaldehyde, Sterile antibiotic discs were placed on MH agar and incubated at 37°C for overnight. Zone of clearance surrounding the discs was measured using a transparent ruler and the diameter was recorded in mm. Tween 80 was used as control. Values are described as mean ± SD of assays performed in triplicate

Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was measured by broth dilution method using Mueller Hinton Broth (MHB).Overnight broth cultures of *E.coli* were adjusted. 20ìl of different concentrations of Cinnamonaldehyde was placed in sterilized test tubes to which contain 100ìl of overnight cultured broth. The tubes were incubated at 37°C for 18–24 h and OD600 values of the cultures were measured, and the lowest concentration that inhibited the bacterial growth was taken as the MIC; the determinations were performed in triplicates. All the MIC tubes (100ìlof culture from each tube) were then used for spreading on Muller Hinton Agar plates for colony counting. The concentration at which no growth was observed was determined as minimal bactericidal concentration (MBC).

Total plate count

The total plate count TPC (aerobic, mesophilic organisms) defines how many aerobic (oxygen-loving), mesophilic (moderate-temperature-loving) micro-organism colonies such as bacteria, yeast and mould fungi will grow in 72 hours on an agar plate that was normed for microbiological testing at a controlled temperature of 30°C.The sample is disintegrated in a standard diluent. The fibre suspension is transferred and incubated. After the incubation time, the numbers of bacterial colonies are counted on the respective plates. The results are expressed separately as the total colony number per gram dry mass of the sample**.**

Determination of fat and protein content

Analyses of protein, fat, were performed for three replicates. Methods used for the estimation were AOAC standard methods [28].

Color Analysis of Sprouts

The colour parameter of sprouts was monitored by Hunter L*, a* and b* values using Hunter Colorimeter. L* (lightness), a* (redness) and b* (yellowness) values were measured for sprouts in three replicates.

Preparation of sprouts

Green gram seeds were purchased from local super market;sprouts were prepared at home at ambient temperature by soaking seeds in three times of water of its seed weight and soaked it for 6-8 hours. Though seeds were small in size it will take less time for soaking. After the proper soaking of seeds, water was drained from seeds. The drained seeds were kept in muslin cloth or sieve and covered using cloth for proper germination for 10-12hrs. Actually germination procedure varies with climatic conditions, as project was undertaken in Chennai due to humid climate seeds were sprouted in open climate without covering it with cloth as it was growing sticky and sour. Then sprouts were kept in water containing cinnamon essential oil prior to using for decontamination.

Table 1. Antibacterial assay

Antimicrobial agent	Micro-organism	Concentration	Zone of inhibition (mm)
E. coli	Cinnamon essential oil	1%	24± 0.8
		1.8%	25± 0.8
		2.55	27± 0.8
		3.5%	29± 0.8
		4%	32± 0.8

Sensory Analysis

The sensory analysis for various samples was conducted for taste, aroma, texture and appearance. The sensory evaluations were conducted on nine point hedonic scale. The panelists were asked to rate the acceptability of the product on a scale of 9 points ranging from 9 to be "like extremely" to 1 to be "dislike extremely".

Sensory was carried out in between semi trained and untrained people for acceptability of product.

RESULT AND DISCUSSION

Isolation of *E.coli* from water samples

Escherichia coli contamination of water has emerged as an important public health concern. Drinking and recreational waters have been linked to human cases of disease Furthermore, *E. coli* can be present in every water source which can causes human illness, so *E.coli* was isolated from drinking water source which was used for germination of seeds, where total plate count was found for *E.coli* was 9.2 x 10^8 CFU.

Antimicrobial assay

Antimicrobial assay was carried out by Disk diffusion method to find out zone of inhibition diameter for cinnamon essential oil purchased from market.

Tabl 2. Different parameters of germination

Sample	Temperature	Seed weight	Amount of water	Time for soaking	Wt. after soaking	Time for germination	Wt. after germination
Green gram	25°C	50g	150ml	6-8hr	80g	8-10hrs	110g

Tabl 3. Total plate count of sprouts of 24 hours

Conc.	Antibacterial effect Colony count						
	0hr	1hr	2hr	4hr	8hr	12hr	24hr
Control	6.5	6.5	6.5	6.5	7.1	7.5	8.5
1%	6.5	6.5	6.3	6.1	5.9	5.9	5.9
1.8%	6.5	6.5	6.2	5.9	5.7	5.7	5.7
2.5%	6.5	6.5	6.1	5.7	5.6	5.5	5.4
3.5%	6.5	5.8	5.5	5.3	5.3	5.2	5.2
4%	6.5	5.4	4.9	4.7	4.7.	4.7	4.7

Table 4. Biochemical and nutritional analysis of control samples (green gram sprout)

Control	Days			
Content	0^{th}	1^{st}	2^{nd}	3^{rd}
Protein (g)	4	3	3	3
Fat (g)	0.6	0.4	0.3	0.2

Table 5. Biochemical And Nutritional Analysis Of Sample Containing Cinnamon Oil

Sample Containing Cinnamon Oil	Days			
Protein(g)	4	3	3	3
Fat (g)	0.6	0.4	0.3	0.2

Table 7. Sensory Evaluation

Attributes	Samples	
	Control	Samples
Taste	9	8.5
Colour	9	8.5
Texture	9	8.5
Appearance	9	8.5
Aroma	9	8.5
Overall Acceptability	9	8.5

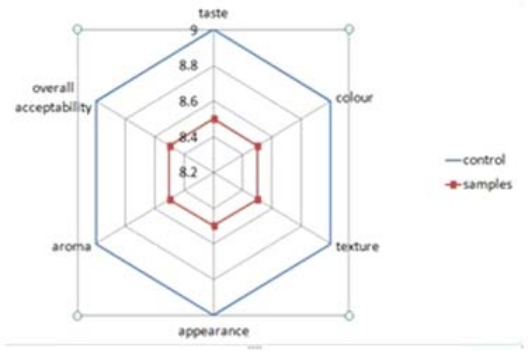

Fig. 1. Total plate count reduction in sprouts after incorporation of antibacterial agents

reduction of *E.coli* contamination as pretreatment for soaking of sprouts and Antibacterial effect study was carried out by doing total plate count after some hours interval.

Effect of antibacterial agent on Biochemical and nutritional content of sprouts

Green gram seeds purchased from market and sprouted and analyzed for protein and fat which shows 3.2g, 0.2g protein and fat respectively, Non significance was observed in content of sprouts after addition of antibacterial agent than control samples.

Table 6. Color Analysis Of Green Gram Sprouts After Addition Of Antibacterial Agents

Sample	L*	a*	b*
Standard	56.64	-21.69	11.05
Control	55.33	-20.32	17.93
1%	54.85	-20.16	18.41
1.8%	53.42	-20.12	17.69
2.5%	53.09	-20.09	19.46
3.5%	52.15	-19.45	17.08
4%	53.69	-19.56	19.13

Germination of green gram

Sprouting is the practice of germinating seeds to be eaten raw or cooked. Sprouts can be germinated at home or produced industrially. They are a prominent ingredient of the raw food diet, some parameters to be maintained while germination.

Effect of Antibacterial agent on sprouts after incorporation of essential oil

Cinnamon essential oil was incorporated in water which used by mixing it with tween 80 for

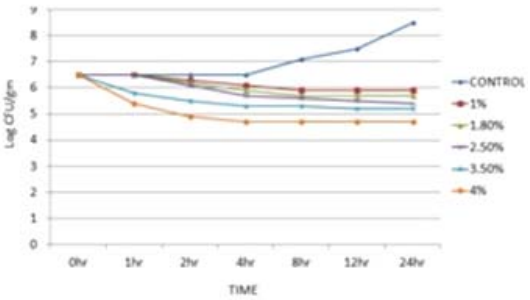

Fig. 2. Sensory analysis diagram

Color analysis of green gram sprouts by hunter colorimeter

The values of color analysis of green gram sprouts after addition of antibacterial agent showed that it has the same color as like control. It shows there is no significant color difference between control and cinnamon oil containing samples.

Sensory analysis

Sensory evaluation was carried out using 9 point hedonic scale. The product was optimized based on sensory analysis with acceptable score 8.5. The test samples were reasonably acceptable

.Sensory analysis of the control sample and the test samples (sprouts pre treated with cinnamon(EO) essential oil) was conducted. The test sample sprouts contained 1%, 1.8%, 2.5%, 3.5% ,4% EO water for pretreatment respectively. Subjects were asked to sample the sprouts and scores were given based on taste, texture, appearance, aroma and overall acceptability.The sensory analysis result showed that the samples which treated with 2.5% of Cinnamon oil among the other oil incorporated sprouts scored more on the analysis. The overall acceptability of the samples containing 2.5% oil was considerably higher than the other test samples and the control.

CONCLUSION

Sprout is the intermediate stage between the seed and plant containing cotyledon having great kind of nutritional contents. It is said that sprouts 'represent the miracle of birth'. They are in the true sense, super foods. They are alkaline, whole, pure, and natural foods. It is inexcusable that though aware of their miraculous effects, sprouts are usually consumed in raw and cooked form, and which cause the chances of human illness due to presence of *E.coli* which came from water source used for germination of seeds. Aim of this project is that to reduce the contamination level to the acceptable level by giving pretreatment of natural antibacterial agent i.e. Cinnamon essential oil. Sprouts are the protein rich products which are used as energy boost, which gives proper nutrition to human and that nutrition was not affected by cinnamon essential oil, so this project indicates that contamination of microorganism can be reduced by some natural potential agents and that not cause any significant effect on quality parameters of sprouts.

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