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THE ESSENTIAL OIL FROM THE SPICES AND HERBS HAVE ANTIMICROBIAL ACTIVITY AGAINST MILK SPOILAGE BACTERIA

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ABSTRACT: *Spices and herbs are usually added to milk to impart a particular flavour and medicinal purposes. Moreover, it is reported that additional spices and herbs extend the shelf life of milk. Contemporary use of essential oils from spices and herbs show promising results against various food spoilage microorganisms. Therefore, the essential oils from the spices and herbs from Zanzibar were used to assess antimicrobial activity against milk spoilage bacteria. The essential oils were extracted using steam distillation by a Clevenger apparatus. Minimum inhibitory concentrations (MICs) were determined by the microdilution method. All the extracts from cardamom, cinnamon, ginger, and lemongrass showed antimicrobial activity against Streptococcus thermophillus, Lactobacillus plantarum, and Escherichia coli. The essential oils exhibited higher antimicrobial activity than gentamycin. The MICs ranged between 0.004 and 0.125 µg/µl. Therefore, spices and herbs added to milk have the potential to inhibit the growth of milk spoilage bacteria.*

KEYWORDS: Milk, Spoilage, Spices, Herbs, Essential Oils, Antimicrobial Activity.

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INTRODUCTION

Milk is an essential source of nutrients for humans and animals. Moreover, being highly nutritious, milk serves as an ideal medium for the growth and multiplication of various microorganisms (Shobana and Naidu, 2000). However, milk spoilage is a problem in many developing countries due to limited cooling storage facilities and an irregular electricity supply (Lore et al., 2005). Therefore, pasteurization is the reliable method used to extend the shelf life of milk.

On the other hand, milk contains a natural enzyme, alkaline phosphatase, with a thermal death value of 4.8°C, which is greater than that of the most heat-resistant non-spore-forming microbes commonly found in milk (Murthy et al., 1993). However, Gram-positive and rod-shaped bacteria such as *Bacillus, Paenibacillus, and Clostridium genera* (Thusitha et al., 2002; Murphy, 2010) develop resistant spores under high temperature. These spores can remain dormant for an extended period until conditions become favourable to germinate into vegetative bacterial cells capable of initiating spoilage even in packed products. In addition, some preservatives, such as potassium metabisulphite and sodium benzoate, are used to extend the shelf life of milk products, such as a non-rennet cheese spread (Dwivedi et al., 2014). Nevertheless, consumers have been consistently concerned about possible adverse health effects caused by chemical preservatives in their foods. Therefore, research is needed to find natural and effective methods to preserve surplus milk (Chen and Hoover, 2003).

On the other hand, several spices and herbs are routinely used in food play a great role against food spoilage microorganisms. That is because spices and herbs contain essential oils which render strong antiviral, antifungal, and antibacterial activities. Similarly, Lutterodt et al. (1999) reported comparable bactericidal and antifungal effects between lemongrass and penicillin. Normally, essential oils contain compounds that are integrated into mouthwash, medicine, and anti-acids (Bhowmik et al., 2012). So far, adding plant-based extracts to dairy and dairy products seem to prolong those products' shelf life (Alenisan et al. (2017). For example, vanillin from vanilla (*Vanilla planifolia*) showed antibacterial activity against *E. coli* and *Listeria innocua* (Fitzgerald et al., 2004). Also, cardamom (*Elettaria cardamomum*) showed promising antibacterial activity against *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella typhimurium* (Agaoglu et al., 2005). Nevertheless, essential oil from cinnamon and thyme reduced the rate of microbial growth in soft white cheese stored for 30 days (Saad and Abdel-Salam, 2015).

Similarly, in our field survey, we found that most households in Zanzibar add spices and herbs such as cardamom, cinnamon, ginger and lemongrass to impart flavour and preserve milk for a short time, up to 24 hours. About a one to one and a half teaspoon equivalent to 5 - 15 g is added to one litre of milk before boiling. Therefore, the objective of this study was to extract essential oils from cardamom, cinnamon ginger, and lemongrass and explore their antimicrobial activities against *S. thermophillus, L. plantarum*, and *E. coli* ATCC 25922.

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MATERIALS AND METHODS

Extraction of essential oils from spices and herbs

The fresh spices and herbs were collected from Kizimbani farms in Zanzibar and transported to the Institute of Traditional Medicine laboratory (ITM) in Dar es Salaam. The procured spices and herbs were packed well and stored in the freezer to avoid the oxidation and evaporation of essential oils, which are volatile. The frozen spices and herbs were weighed into the round flask, and essential oils were extracted by steam distillation using the Clevenger apparatus. The temperature was maintained at 60˚C to avoid burning the extracted materials to get clear and maximum yield. Then, essential oils were aseptically collected and stored in sealed vials in a fridge.

Multiplication of bacteria cells

The bacterial strains of *Lactobacillus plantarum* and *Streptococcus thermophillus* were obtained from Food Science and Technology laboratory at the Sokoine University of Agriculture, Morogoro, Tanzania, whereas *E.coli* ATCC 25922 was obtained from ITM. The strains of bacteria were sub-cultured in nutrient broth and soy agar. Every 5 ml of nutrient broth into test tubes was inoculated with bacteria strains for three hours to activate the bacteria cells. After that, 0.5 ml of bacteria suspension was poured onto plates with agar, spread over using sterile swab sticks and incubated at 35°C overnight. Moreover, each essential oil was cultured and incubated under the same condition as the samples to detect if there was a contamination.

Antimicrobial test of essential oils

A two-fold serial dilution method was carried out to determine the antimicrobial activities of the essential oils. Each strain of the bacterial suspensions was prepared in sterile normal saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. Exactly, fifty microliters of the broth were drawn into each well of the sera culture plate, followed by adding 50 µl of the essential oil in each first well of the row. Two-fold serial dilution was made from the first well to the last well in the row, and the last 50 µl was discarded. After that, 50 µl of the test organisms were added to each well. A test of all the treatments was performed in triplicate to increase validity. The plates were covered, marked, and incubated at 37ºC overnight. Moreover, positive and negative controls were used, respectively, gentamycin and soy peptone broth. Two hours before reading the results, 20 µl of the 0.2% nitrotetrazoleum (NIT indicator) were added to the culture plate and incubated to observe typical purple colouration. The minimal inhibitory concentrations (MIC) were recorded as the last well in a row with no colour change (Andrews, 2011; NCCLS, 2000).

Data Management and Analysis

The data were analyzed using MIC standard value classification (Table 1) as described by Aligiannis et al. (2001) and Sartoratto et al. (2004).

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RESULTS AND DISCUSSION

Extraction of essential oils

A huge mass of foams was observed during cardamom and cinnamon oil extraction in the flask. According to Eloff (1998), the foam might be due to phyto constituent bioactive components such as saponin present in spices and herbs. The oil from cinnamon formed an emulsion with hydrosol, which separated into layers after the extract was left to cool. The essential oil colour was slightly yellow for cinnamon, green-yellow for lemongrass, and slightly yellow for ginger, while cardamom was clear colourless. Lemongrass oils were characterized by sharp odour and higher irritability on the skin, while ginger oil was less irritative. The irritating characteristic of essential oils is mostly due to their lower pH (Isam et al., 2009).

Among all the oil extracts, cardamom had the highest yield (5.3%), followed by cinnamon (1.3%), lemongrass (0.4%), and ginger (0.4%) (Table 2). Considering that storage conditions can influence the yield of essential oils from the spices (Agaoglu et al., 2005), the high yield from cardamom could be due to its natural pod, which protects the evaporation of its essential oil.

The antimicrobial activity of the essential oils

The MIC of essential oils required to inhibit the growth of microorganisms are shown in Figure 1. The essential oils from cardamom, lemongrass, cinnamon, and ginger demonstrated a stronger antimicrobial activity (< 0.5 µg/µl) than gentamycin in all tested bacterial strains. Gentamycin showed the weakest antimicrobial activity against *S. thermophillus* (5 µg/µl), followed by *L. plantarum* (2.5 µg/µl) and *E. coli* ATCC 25922 (0.312 µg/µl).

On the one hand, essential oils from cinnamon, cardamom and lemongrass demonstrated a comparable antimicrobial activity (0.063 µg/µl) against *L. plantarum*, whereas the lowest concentration (0.016 µg/µl) was demonstrated from ginger. Conversely, the highest concentration of essential oils from ginger (0.125 µg/µl) was required to inhibit the growth of *E. coli* ATCC 25922. On the other hand, the lowest MIC to inhibit the growth of *E. coli* ATCC 25922 was observed at lemongrass (0.008 µg/µl), followed by cinnamon (0.031 µg/µl) and cardamom (0.063 µg/µl). Nevertheless, cinnamon and ginger exhibited the lowest MIC against *S. thermophillus* (0.04 µg/µl), followed by cardamom (0.016 µg/µl) and lemongrass (0.063 µg/µl).

Gram-negative bacteria, *Escherichia coli*, are more resistant to the chemical than Gram-positive bacteria (Street and Staros, 2014). However, the *L. plantarum* and *S. thermophillus* have also shown high antimicrobial resistance to gentamycin. Therefore, essential oils from spices and herbs have shown the potential to inhibit the growth of a range of bacteria. For example, Ates et al. (2003) found that alcoholic extract from cinnamon inhibited the growth of *Bacillus megaterium* and *Enterococcus faecalis*. Also, Mahady et al. (2005) reported the antibacterial activity of the essential oils from cardamom and cinnamon against *Escherichia coli*, *Streptococcus oralis*, *S. anginosus*, *S. intermedius*, *S. sanguis*, and *Enterobacter aerogenes*. Further, Saad and Abdel-Salam (2015) observed that a minimum of 0.03% of the essential oil from cinnamon was required to inhibit the growth of coliforms in soft white cheese stored for 30 days.

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Essential oils may contain saponins, tannins, alkaloids, and flavonoids, naturally occurring compounds in medicinal plants that may possess bactericidal, pesticidal, or fungicidal activities of the spices and herbs (Eloff, 1998; Rios and Recio, 2005). Moreover, the pH of many essential oils contributes to a synergistic effect with the bioactive components present in spices and herbs (Hamza et al., 2009). However, MIC does not describe the presence, type or location of the antimicrobial action **(**Street and Staros, 2014). Therefore, the activity of essential oil as a natural preservative depends upon the type of spice, the concentration of oil used, test medium, virulence factor or resistance of a microorganism to a given chemical compound, and initial microbial load (Selim, 2010). Moreover, detailed knowledge about the mode of action and the effect on food matrix components and their antimicrobial properties is required (Andrews, 2011). Furthermore, the effect of the essential oil can be bactericidal or bacteriostatic in action (Hamza et al., 2009). Therefore, a minimal bactericidal concentration test should be determined to verify whether the exhibited antimicrobial effect was bactericidal or bacteriostatic.

To shed light on inducing antimicrobial activities in one litre of milk, about 63 ml of each essential oil from cardamom, cinnamon and ginger and 16 ml of lemongrass are required to inhibit the growth of *L. plantarum*. For the inhibition of *E. coli*, at least 63 ml was required from cardamom, 31 ml from cinnamon, 125 ml from ginger and 8 ml from lemongrass. Moreover, to inhibit the growth of *S. thermophillus* required, 16 ml of essential oils from cardamom, 4ml from cinnamon, 4ml from ginger, and 63 ml from lemongrass. Therefore, the amount used to impart flavour in milk is insufficient to inhibit the in-vitro growth of studied microbes. However, the milk is normally boiled with spice or herbs before consumption. Therefore, the effect of the combination between essential oils and boiling temperature and the time is recommended for further studies.

CONCLUSION

The spices and herbs added to milk for flavour, and medicinal use can inhibit the in-vitro growth of milk spoilage bacteria. The essential oils from spices and herbs had higher antimicrobial activity than gentamycin. However, the established minimum inhibitory concentration for each essential oil on a given microorganism require further attempts to test the effect of these oils, whether they are bactericidal or bacteriostatic. Practically, the amount of essential oils from spices and herbs added in milk is insufficient to exhibit antimicrobial activity. Therefore, the non-essential oil components from the added spices and herbs are to be studied and identified for their chemical structures and antibacterial characteristics to expand their database as potential natural milk preservatives.

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ANNEXE 1. LIST OF TABLES

Table 1: the minimum inhibitory (MIC) standard value classification

MIC (µg/ µl)	Classification of antimicrobial activity of drugs
0.050 – 0.500	Strong antimicrobial activity
0.600 – 1.500	Moderate antimicrobial activity
MIC > 1.500	Weak antimicrobial activity

The vaalues were adappted from Aligiannis et al. (2001) and Sartoretto et al. (2004). The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after the overnight incubation (Andrews, 2011).

Table 2: Yield of extracted essential oils

Spice	Weight used (g)	Volume of extract (ml)	Yield (%)
Cardamom	91.86	5.8	6.31
Cinnamon	522.64	8.6	1.65
Ginger	843.89	3.2	0.38
Lemongrass	1432.01	1.8	0.13

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ANNEXE 2. LIST OF FIGURES

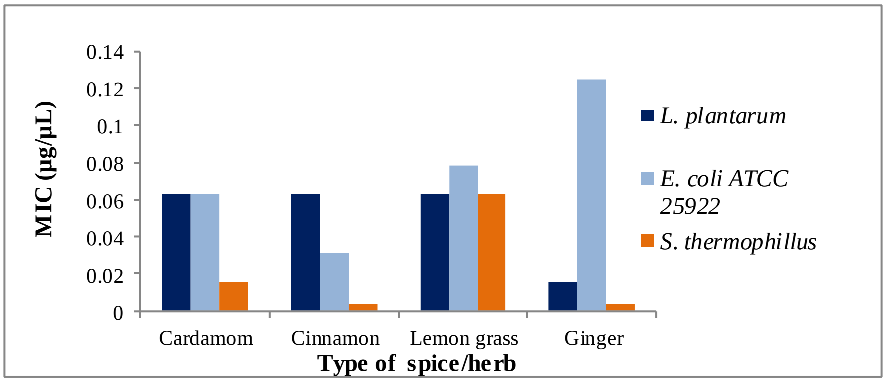

Figure 1: Minimum inhibitory concentration (MIC) values to inhibit the growth of milk spoilage bacterial strains

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