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Original article

Impact of *Cinnamomum verum* against different *Escherichia coli* strains isolated from drinking water sources of rural areas in Riyadh, Saudi Arabia

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ABSTRACT

Objectives: The present study was aimed to evaluate the antimicrobial activity of aqueous, methanolic and essential oil extracts of *Cinnamomum verum* (cinnamon) against two waterborne pathogenic strains isolated from bottled, tap and wells' water from the rural areas of Riyadh, Saudi Arabia.
Methods: The water samples were drawn from different sources and two strains of *Escherichia coli* were isolated, purified and confirmed using the MALI-TOF-MS technique. The *C. verum* extraction was carried out using different solvents and essential oil by hydrodistillation according to the standard methods. The antimicrobial potential was evaluated using a well-diffusion assay and percentage inhibition was calculated.
Results: The two isolates were identified as *Escherichia coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB by MALDI-TOF-MS. Aqueous, methanolic and oil extracts of cinnamon were tested for their inhibitory activity against the selected strains using the well diffusion method. *E. coli* strains were more sensitive towards the essential oil extract with the inhibitory zone of 4.5–5.2 cm rather than the aqueous and methanolic extracts. Analysis of cinnamon essential oil by GCMS showed the presence of Cinnamic acid, Cinnamaldehyde and 3-Allyl-6-methoxyphenol in the essential oil.
Conclusion: The results indicated the possible potential of *Cinnamomum verum* essential oil and extracts in the management of *E. coli* from different water sources. Possibly, the bioactive compounds including cinnamic acid derivatives may responsible for the bioactivities of the plant.

1. Introduction

Waterborne disease is a universal burden that is determined to cause more than two million deaths per year, including diarrhoea, gastrointestinal diseases and systemic illnesses (WHO, 2015; Bitton, 2014). Causes of waterborne diseases take place by ingestion of microbes, airborne transmission or direct contact with viruses, bacteria, protozoa and helminths found in contaminated water (Leclerc et al., 2002). Unfortunately, more than 800 million people are under the risk of drinking polluted water and they do not even have access to clean and drinkable water (Ramírez-Castillo et al., 2015). As reported by (WHO, 2008), enteric pathogens have developed a gradual increase in their antimicrobial resistance which is characterized as a worldwide major concern. Also, as in the report of WHO in 2015, the decision-makers have noted that by improving the water quality; we could save millions of people around the world. The improvement of bacterial resistance to currently available antibiotics is indeed needed to search for new antibacterial agents. Gram-negative bacteria such as *E. coli* is present in the human intestine and causes infantile diarrhoea, lower urinary tract infection, coleocystis or septicemia (Surbatovic et al., 2015).

Cinnamon species bark is considered as one of the most important species used all over the world for traditional medicine (Błaszczyk, et al., 2021). It belongs to kingdom Plantae, Division: Magnoliophyta and belongs to the Lauraceae family, representing 82.5% of Cinnamon bark total composition (Rao and Gan, 2014; Paliwal et al., 2018). Cinnamon consists of unique components including cinnamaldehyde, cinnamic acid, cinnamate and essential fatty acids such as Eugenol, L-borneol, L-bornyl acetate –Eneroli-

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dol, Terpinolene which give its spicy taste and fragrance (Wariyapperuma et al., 2020). The major components of Cinnamon essential oil are *trans*-cinnamaldehyde or cinnamaldehyde (Zhang et al., 2016). One of the applications of cinnamon in the food industry, its addition to chewing gum as a bad breath removal because of its refreshing effects, (Rao and Gan, 2014). Cinnamon is used as a coagulant as it prevents bleeding and also, it increases blood circulation (Mahmoodnia et al., 2017). Cinnamon has also been used as tooth powder to treat toothaches- dental problems, oral microbiota (Zouheyr et al., 2014). Also, they respond to free radicals and alleviates metabolic diseases of humans and other animals due to the presence of Eugenol and linalool oils (Mohamed et al., 2020). Cinnamon acts as an anti-inflammatory agent due to the presence of flavonoid compounds such as gossypin, gnaphalin, hesperidin, hibifolin and oroxindin which suppress the expression of inducible nitric oxide synthesis (Cho et al., 2013). Using this mechanism, Cinnamon is considered as a nitric oxide producer in the central nervous system which decreases the lipopolysaccharide-induced tumor necrosis in the serum (Han and Parker, 2017).

Cinnamon oils are well known for their inhibitory potential activity against many pathogenic bacteria such as *Pseudomonas aeruginosa*, *E. coli and staphylococcus aureaus*, and also against fungal pathogens such as *Candida albicans*, *Torulopsis utilis, Saccharomyces cerevisiae and Schizosaccharomyces pombe* (Narayanankutty et al. 2021a; Baker and Grant, 2018; Abd El-Hack, et al., 2020). It is revealed that essential oil from Cinnamon is more effective than its aqueous extract as an antibacterial agent (Parthasarathy and Thombare, 2013; Syafiq et al., 2021) as it exerted potent inhibitory effects against *E. coli* and *S. aureus* with a diameter of inhibition zone values of 19.2 and 28.7 mm, respectively. Liaqat et al. (2017) stated that Cinnamon has an inhibition effect against wide Gram-negative and Gram-positive bacteria to reach 12.17–29.5 mm suggesting a high antibacterial activity. Thus, we herein aimed to detect the inhibitory activity of cinnamon oil extract against the growth of pathogenic *E. coli* strains.

2. Materials and methods

2.1. Microorganisms and media

Two isolates were isolated from drinking water bottled, tap and wells' water from rural areas in Riyadh, Saudi Arabia; namely *Escherichia coli DSM 1103 QC DSM and E. coli MB 11,464 1 CHB*. Media used: all media were prepared as described by (APHA, 2005). Violet red bile lactose (VRBL) Agar was prepared for *E. coli* sp. isolation. It has the following composition (g/l): Lactose 10.0, Peptone 7.0, Yeast extract 3.0, Sodium chloride 5.0, Bile salts mixture 1.5, Neutral red 0.03, Crystal violet 0.002, Agar 15.000 with pH adjusted to 7.4 ± 0.2 at 25 °C. For isolate maintenance, Nutrient agar medium (g/l) was prepared. It has the following composition: meat extract 3, peptone 5, pH 7. For testing the inhibitory activities of cinnamon essential oil, Muller and Hinton agar with a composition of (g/l) beef, dehydrated infusion 30.0, casein hydrolysate 17, Starch 1.5, agar 15.0 with pH adjusted to 7.2 ± 0.1 at 25 °C was prepared.

2.2. Preparation of cinnamon extracts

a. Aqueous Extract: Ten Gram of dried and crushed cinnamon barks were soaked in 100 ml of sterilized distilled water for 6 h. at 50 °C. filtration was done every two hours using eight layered muslin cloth then centrifuged at 10000 rpm for 15 min. The supernatant was then collected and concentrated through evaporation at 40 °C to the final volume. The aqueous extract was sterilized by filtration. For aqueous extract maintenance, it was stored at 4 °C in airtight bottles for further studies. (Parekh et al., 2005).
b. Solvent extract: Ten grams of dried and crushed cinnamon barks was added to 100 ml of methanol solvent 90 % (v/v) then, kept on a rotatory shaker at 120 rpm for 24°C (Lab-Line Orbital Shaker- USA Lab Equipment) for 24 h. filtration and maintenance were carried out as previously described by (Parekh et al., 2005).
c. Essential oil of cinnamon: The cinnamon essential oil was prepared by extraction of aromatic oil unit. The extraction was done by hydro distillation process following the methods of Narayanankutty et al., (2021b).

2.3. Standard inoculum

The standard inoculum was prepared by inoculating 5 ml of peptone water by 3–5 single colonies of the selected isolate and incubated at 37 °C for 24 h. Thereafter, the Optical density (O.D.) of the grown culture was adjusted to 0.06–0.8 using the spectrophotometer at 625 nm which is equivalent to ($14x10^6$ CFU/ml) (Ebrahim et al., 2018).

2.4. Inhibitory effect of Cinnamomum verum extracts against E. Coli sp. Using well diffusion test

Inhibitory activity of cinnamon (aqueous, methanolic and oil) extracts was tested individually using well-diffusion method as recommended by (NCCLS, 1993). Muller Hinton agar medium was poured into petri dishes and inoculated with 1 ml of *E. coli* isolates ($14X10^6$ CFU/ml) using the spreading technique. Agar wells were made using a sterilized 7 mm corkborer and filled with 100 μl of the tested cinnamon essential oil. Incubation of petri dishes was done at 37 °C for 24 h. All experiments were carried out in triplicates. The cinnamon inhibitory activity was expressed as the inhibition zone diameter's means (NCCLS, 1993; Narayanankutty et al 2021c).

2.5. Effect of different concentrations of cinnamon essential oil against E. Coli sp. By well diffusion method

Serial concentrations of cinnamon oil were prepared by emulsifying (30–40–50–60–70–80 % v/v) in 2 % of tween 80. These concentrations of cinnamon oil were tested for their inhibitory activity against *E. coli* isolate sp. The inoculum was prepared by inoculating the nutrient broth by 3–5 colonies of *E. coli* and incubated for 24 h. at 37 °C. Inhibitory activity was determined as described above. All experiments were carried out in triplicates and the cinnamon inhibitory activity was expressed as the inhibition zone diameter's mean (Mahdi et al., 2018).

2.6. GCMS analysis of essential oil of Cinnamomum verum

A Hewlett Packard gas chromatograph (HP6890) connected to a VG Analytical 70250S mass spectrometer with an HP5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). In this system, helium is used as the carrier gas and the flow rate is 1 ml/min. The oven temperature range is adjusted from 50° C in 5 min to 280° C, and the oven temperature increase program is 40° C / 5 min, and finally, the temperature is kept isothermal for 5 min. Inject 1 μl of cinnamon oil sample in split mode. GC–MS detection was performed using a 70-eV ionization energy electron ionization system. A scan rate of 0.6 s (cycle time: 0.2 s) was applied, covering a mass range from 35 to 600 amu. The identification of the essential oil

compounds was based on the comparison of retention time and mass spectra of the homolog's series of (C4-C28) with data generated under identical experimental conditions (Adams, 2017).

2.7. Identification of pathogenic waterborne isolates by MALDI-TOF MS

MALDI-TOF-MS was used to identify the *E. coli* isolates by picking up a single colony of the selected isolate and transferring it directly using wood backs on MALDI target plate at ambient temperature (25 °C) until dry. Overlaying of samples was done by adding 1 μl of Bruker HCCA solution to all MALDI plates and leaving them until drying.

2.8. Statistical analysis

The determination coefficient (R^2) was calculated according to Microsoft office Excel 2016 package.

3. Results

3.1. Isolation and identification of E. Coli isolates by MALDI-TOF MS

VRBL agar medium was prepared to isolate *E. coli* isolates from drinking water in the rural areas in Riyadh, Saudi Arabia, which represent 35 % of the bacterial waterborne microorganisms. The medium was inoculated using 1 ml of water sample then incubated at 37 °C for 24 h. Single colonies with lactose-fermenting appearance (pink colonies) were picked up and sub-cultured into nutrient agar for maintenance. The results indicate the high score of the two selected *E. coli* isolates ranged from 2.41 and 2.46 identified as *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB (Table 1).

Table 1
MALDI-TOF-MS score values for the selected waterborne pathogenic isolates from drinking water from rural areas in Riyadh, Saudi Arabia.

Isolate no.	Isolate source	Score Value	Suggested strains
3A	Bottled and tap water	2.41	*E. coli* MB 11,464 1 CHB
40C	wells' water	2.46	*E. coli* DSM 1103 QC DSM

3.2. Inhibitory effect of Cinnamomum verum against E. Coli strains

Different (Aqueous, methanolic and oil) cinnamon extracts were prepared and tested for their inhibitory activity against *E. coli* strains using the well-diffusion method. The current results show that the oil extract of cinnamon inhibited the growth of *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB rather than the aqueous and methanolic extracts that indicates the high sensitivity of *E. coli* strains towards the oil extract with inhibition zone 4.5 cm and 5.2 cm for *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB (Figs. 1 & 2).

3.3. Effect of different concentrations of cinnamon essential oil against E. Coli DSM 1103 QC DSM and E. Coli MB 11,464 1 CHB strains

Different concentrations of cinnamon oil extracts were prepared from 30 to 80% (v/v) and tested against *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB strains. The results show that oil concentration of 80% (v/v) had the highest inhibitory activity on E. coli strains followed by the 70% and 60% (v/v) concentrations, respectively (Fig. 3). Determination coefficient of R^2 = 0.9449 and 0.9667.

3.4. Chemical components of cinnamon essential oil extract

The chemical composition of cinnamon essential oil was done using GCMS analysis (Table 2). The major constituents include Propylene glycol, Linalool, Cinnamaldehyde, 3-Allyl-6-methoxyphenol, Methyl *cis*-cinnamate, Ethyl- cinnamate, Cinnamic acid, phenethyl ester, and Benzyl cinnamate. Cinnamaldehyde was found to have the highest percentage (24.42%) followed by cinnamic acid (20.93 %) and 3-Allyl-6-methoxypheno (m-eugenol) (18.55%).

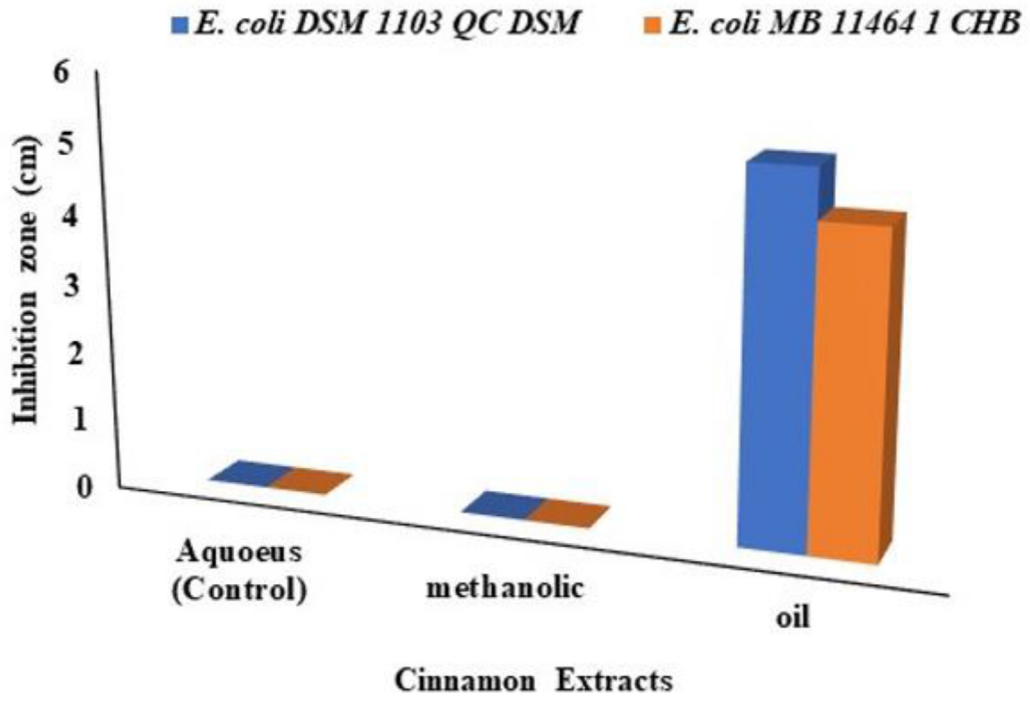

Fig. 1. Antimicrobial activity of different cinnamon extracts against *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB strains.

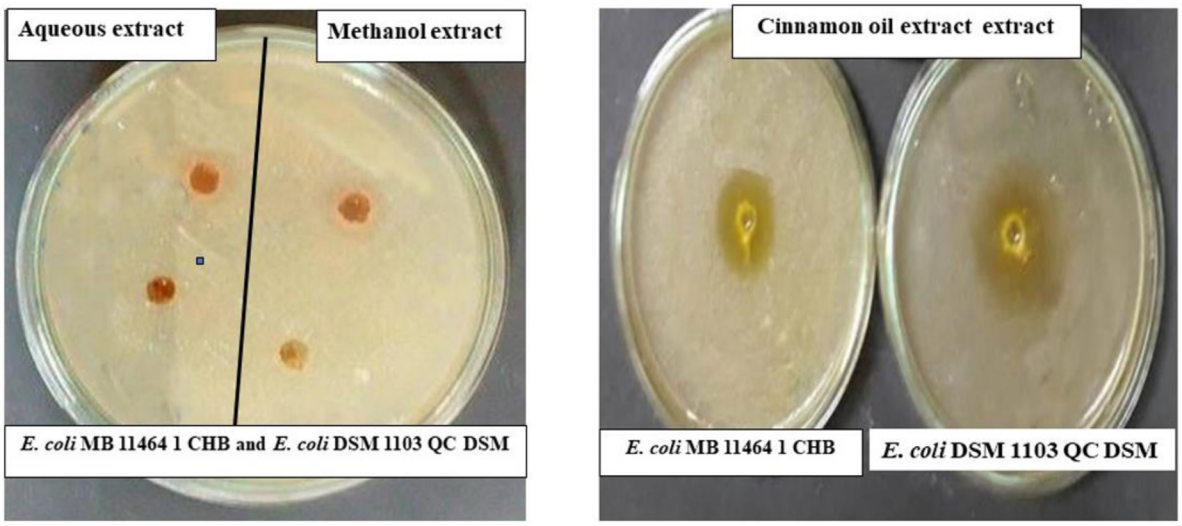

Fig. 2. Antimicrobial activity of different cinnamon extracts against *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB strains using well diffusion method.

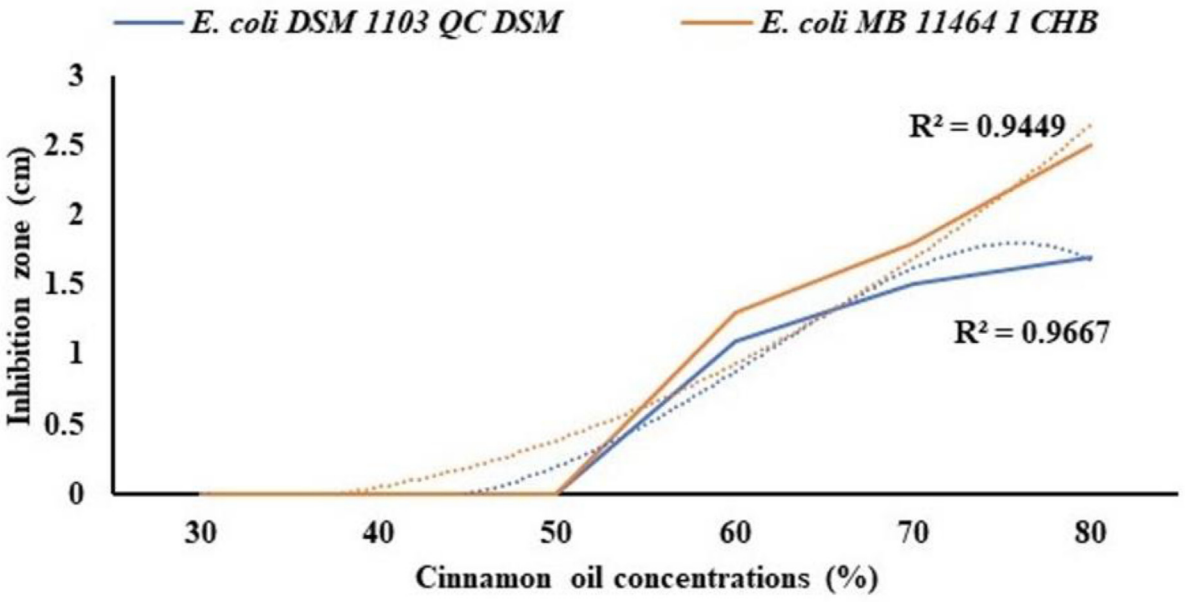

Fig. 3. Different concentrations of cinnamon oil extract against *E. coli* DSM 1103 QC DSM and *E. coli* MB 11,464 1 CHB strains.

Table 2
Chemical constituents (%) of cinnamon oil detected by GC–MS.

Sample	Retention time	%	Compound name
1	4.7443	7.94	Propylene Glycol
2	8.0698	1.05	Linalool
3	10.0979	24.42	Cinnamaldehyde
4	10.7863	18.55	3-Allyl-6-methoxyphenol
5	11.051	4.42	Methyl *cis*-cinnamate
6	11.7341	1.04	Ethyl cinnamate
7	12.4331	21.03	Cinnamic acid, phenethyl ester
8	12.4967	20.93	Cinnamic acid, phenethyl ester
9	16.4205	0.57	Benzyl cinnamate

4. Discussion

Waterborne disease is a worldwide burden that is causing the death of more than two million people per year. A lot of waterborne associated disease like diarrhea, gastrointestinal diseases and systemic illnesses are invading many regions around the world (WHO, 2015). Unfortunately, more than 800 million people are at risk of waterborne diseases (Ramírez-Castillo et al., 2015). Cinnamon composition of Propylene Glycol, Linalool, Cinnamaldehyde, 3-Allyl-6-methoxyphenol, Methyl *cis*-cinnamate, Ethyl- cinnamate, Cinnamic acid, phenethyl ester, and Benzyl was similarly to El Atki et al., (2019) and Wong et al., (2014) who found that cinnamic acid, phenethyl ester are found to have the highest component of cinnamon with a ratio of 45%. Previous studies have also indicated the higher composition of cinnamic acid derivatives in the Cinnamomum essential oil (Narayanankutty et al., 2021a). Moreover, the presence of antimicrobial agent, cinnamic acid exhibits different antimicrobial activities at its higher concentrations. Also, Becerril et al., 2012 found that cinnamon essential oils inhibited the growth of *Staphylococcus aureus*. Adams et al., 2019 found that cinnamon

essential oil has a great effect on combating *E. coli* growth with an inhibition zone of 5.1 mm when oil is applied with a concentration of 2%.

Our results contributed to the presence of the antimicrobial agent, Cinnamic acid which exhibits different antimicrobial activities at its high concentrations as reported by El Atki et al., (2019). The determination coefficient of R^2 = 0.9449 and 0.9667 confirmed that the cinnamon oil extract inhibited the *E. coli* growth significantly. Our results about the ability of cinnamon essential oils to inhibit the growth of *Staphylococcus aureus* were similarly to Becerril et al., 2012. Cinnamaldehyde was found to have the highest percentage (24.42%) and followed by cinnamic acid (20.93 %) and 3-Allyl-6-methoxypheno (m-eugenol) (18.55%). Similar results were found by Wong et al., (2014) who stated that cinnamic acid, phenethyl ester is found to have the highest component of cinnamon with a ratio of 45%.

In conclusion, the inhibitory activity of cinnamon oil extracts showed a high sensitivity activity against *E. coli* isolated from drinking water in the rural areas in Riyadh, Saudi Arabia rather than the aqueous and methanolic extractions. The results open the door for more researches on the biological ways of treatment of water and encourage the local scientists to participate in such an important field.

5. Conclusion

The study concludes that Cinnamon derived molecules are important antibacterial agents and are capable of limiting the population of *E. coli*. Hence, the plant may be further used for the isolation of bioactive compounds and also can be employed for preventing water-borne bacterial diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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