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Chemical composition, antimicrobial activities and TLC profile of different bark extracts of *Cinnamomum zeylanicum*

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Abstract

The bark of cinnamon (*Cinnamomum zeylanicum*) is commonly used as spice and has also been widely employed in the treatment and prevention of diseases. The aim of the present study is to assess the antimicrobial activity of the 96% ethanolic, petroleum ether, chloroform and methanolic extracts against four standard bacterial species, two Gram-negative bacteria, (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two standard fungal species (*Candida albicans*, *Aspergillus niger*). This study also aimed to investigate the chemical composition of the active extract and to determine the thin layer chromatography profile of different bark extracts of the plant. The results showed that all extracts exhibit significant antimicrobial activity against the tested organisms and the petroleum ether (PE) extract showed the strongest activity and was subjected to GC-MS analysis. The petroleum ether extract was composed of 99% (E) - Cinnamaldehyde which was isolated by preparative TLC. The antimicrobial activity of *C.zeylanicum* most probably due to the presence of (E) – cinnamaldehyde, a known antimicrobial natural product.

Keywords: *Cinnamomum zeylanicum*, Antimicrobial activity, Cinnamaldehyde, GC- MS analysis.

1. Introduction

Infectious diseases are disorders caused by microorganisms — such as bacteria, viruses, fungi or parasites. Many organisms live in and on our bodies. They are normally harmless or even helpful, but some organisms under certain conditions may cause disease [1]. Infectious diseases can be transmitted from person to person or via bites of insects or animals. Also ingestion of contaminated food and water can lead to infectious disease [1]. Each infectious disease has its own specific signs and symptoms. The main treatment of infectious diseases is the chemicals which include antibiotics, antifungal and antiviral agents. But, recently many antibiotics have failed to discourage the growth of many bacteria that have ability to transmit and acquire resistance to drugs. Thus infections with these bacteria are associated with high morbidity and mortality especially in immunocompromised patients. In addition many researches have established the side effects of overuse and misuse antibiotics which can harm vital organs like liver and kidneys [2]. The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug resistant microbes and to reduce the harm cause by antibiotics.

Cinnamon (*Cinnamomum zeylanicum*) is a small evergreen tree which is native to Sri Lanka, southeastern India, Indonesia, South America, and the West Indies [4]. The main constituents of cinnamon bark is cinnamon oil which contains mainly cinnamic acid, cinnamaldehyde and cinnamic alcohol. Cinnamaldehyde is the most prevalent one with concentration of 6,000 – 30,000 ppm [5]. Cinnamon has been used to treat diarrhea and other gastrointestinal problems. It has traditionally been used to treat toothache and fight bad breath and its regular use is believed to treat common cold [6]. It is also believed to possess an antioxidant [6], antiulcer [6] anti-inflammatory, antidiabetic and hypolipidemic effects.

2. Materials and Methods

2.1 Plant and extraction procedure

Cinnamon bark was purchased from Yarol Company in Omdurman. The plant materials were grinded and then extracted using 96% Ethanol in Soxhlet apparatus for 6 h. Another sample from powdered cinnamon bark was extracted successively with petroleum ether 40-60 °C, chloroform and methanol, respectively.

The extracts were evaporated to dryness and finally stored in refrigerator at 4 °C for the next tests. The percentage yields were calculated.

2.2 Screening of antimicrobial activities

2.2.1 Preparation of the media

Nutrient agar powder (5.64 g) was weighed and dextrose powder was weighed and then dissolved in 100 ml of distilled water (for fungal growth). The media was then placed in the autoclave at 121 °C, 15 lbs pressure for 15 minutes for sterilization. The media (20 ml) was poured in a Petri dish aseptically and 0.2 ml of the intended microorganism, which is already prepared in normal saline and kept in fridge, was added at the same time. The media was left for 1 hour. After media become solid 2 holes were made in each plate using cork borer, 10 mm in diameter (The cup-plate agar diffusion method [7]).

2.2.2 Antimicrobial test of the plant extracts

Different extracts (0.2 g) were dissolved in 2ml of a suitable vehicle (methanol, petroleum ether or methanol: petroleum ether 2:1) and were added to the plates, then left for half an hour for better diffusion and then incubated at 37 °C for bacteria and 30 °C for fungi for 18 hours. The zone of inhibition was then measured.

2.2.3 Minimum inhibitory concentrations (MICs) of petroleum ether extract (exhibits the highest antimicrobial activity)

The agar diffusion method was used, after the media was prepared as described above. The extract (0.2 g) was weighed and then dissolved in 4 ml of the suitable solvent to give the concentration of 50% and then serial dilution was made to form the concentration of 25%, 12.5%, 6.25%, 3.125%, 1.5625%, 0.78125% and 0.39%. Inhibition zone was measured.

2.2.4 TLC analysis of petroleum ether extract

The extract (0.3 g) was redissolved in 3 ml of its mother solvent. The sample was applied on to TLC plate and different solvent systems, petroleum ether: diethyl ether: glacial acetic acid, (8:20:1), toluene: ethyl acetate in a ratio of 70:30 and 93:7, were used in order to determine the best suitable solvent for separation. Detection of the spots was carried out by mean of day light, UV lamp and iodine vapor. Rf values were then measured.

2.2.5 GC-MS analysis of petroleum ether extract

PE extract sample was analyzed by GC-MS. Sample (1 µL) was injected into a Varian CP 3800 gas chromatograph coupled with Varian 4000 MS and fitted with a 30 m x 25 µm x 25µm DB-5 capillary column. The carrier gas was Helium and was set to maintain a capillary flow of 1 ml/min. The initial temperature of 40 °C was held for 3 min followed by an increase of 2 °C /min to 150 °C, then 1.5 °C/min to a final temperature of 280 °C.

3. Results and Discussion

3.1 Percentage yields of the extracts

Table 1 showed that the highest percentage yield was occurs when methanol was used. This may indicates that most of the C. zeylanicum bark constituents are of polar nature.

Table 1: Percentage Yields of the different extracts of Cinnamon bark

| Extracting solvent | Extractable matter, % |
|--------------------|-----------------------|
| 96% ethanol | 10.44 |
| PE | 2.21 |
| Chloroform | 1.23 |
| methanol | 3.89 |

3.2 Antimicrobial activity screening

The different extracts of Cinnamon bark were showed variable antimicrobial activities and Figure 1 clearly showed that the petroleum ether extract exhibit highest antimicrobial activity followed by the chloroform extract and finally the methanolic extract.

Table 2: The average zone of inhibition, in mm, of PE, chloroform and methanolic extract.

| Microorganism | <i>E. coli</i> | <i>Pa</i> | <i>Sa</i> | <i>Bs</i> | <i>Ca</i> | <i>An</i> |
|-----------------|----------------|-----------|-----------|-----------|-----------|-----------|
| Petroleum ether | 29.5 | 30.5 | 29.5 | 32.5 | 36.5 | 39.5 |
| Chloroform | 22.5 | 23.5 | 19.5 | 21.5 | 27.5 | 28.5 |
| Methanolic | 20.5 | 19.5 | 20.5 | 21.5 | 21.5 | 25.5 |

* *Pa* (*Pseudomonas aeruginosa*), *Sa* (*Staphylococcus aureus*), *Bs* (*Bacillus subtilis*), *Ca* (*Candida albicans*), *An* (*Aspergillus niger*)

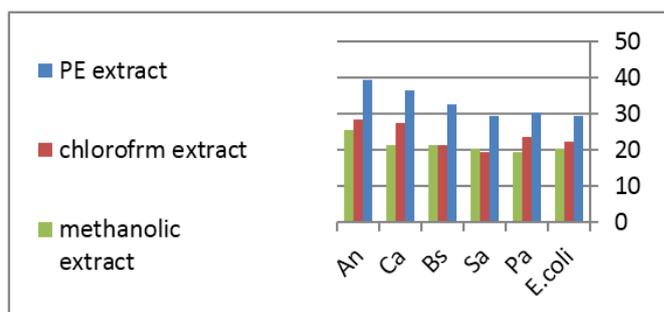


Fig 1: Comparison of the average zone of inhibition, in mm, between PE, chloroform and methanolic extract.

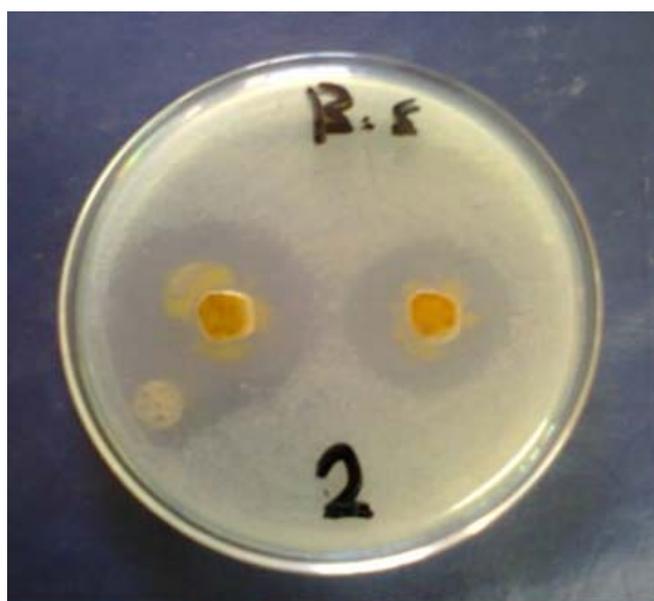


Plate 1: Bacillus subtilis



Plate 2: *Pseudomonas aeruginosa*



Plate 5: *Aspergillus Niger*

Fig 2: Zone of inhibition exhibited by different extracts



Plate 3: *Escherichia coli*

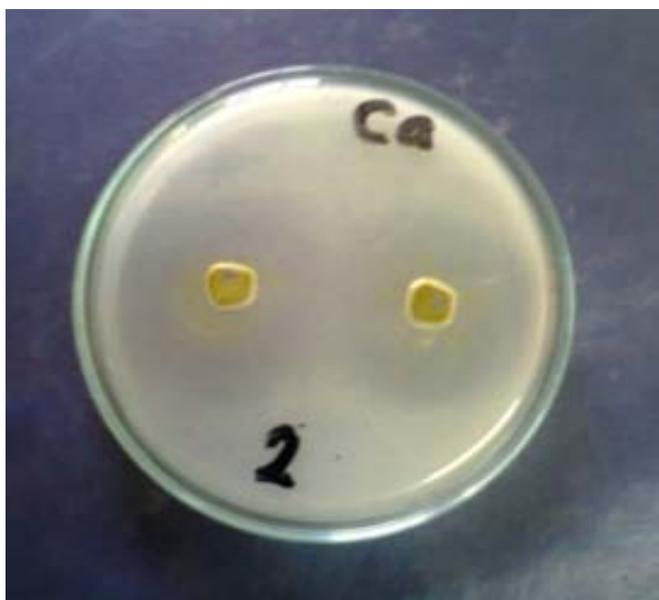


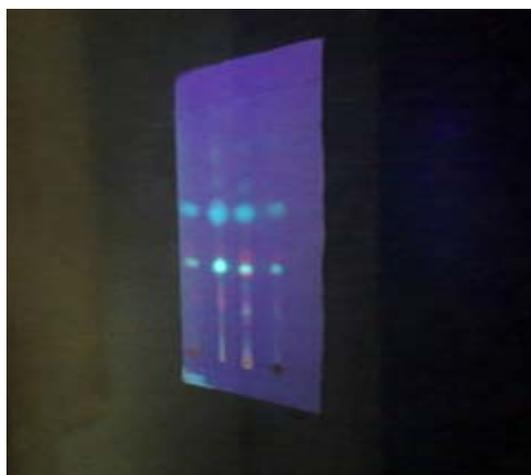
Plate 4: *Candida albicans*

3.3 TLC profile of petroleum ether extract

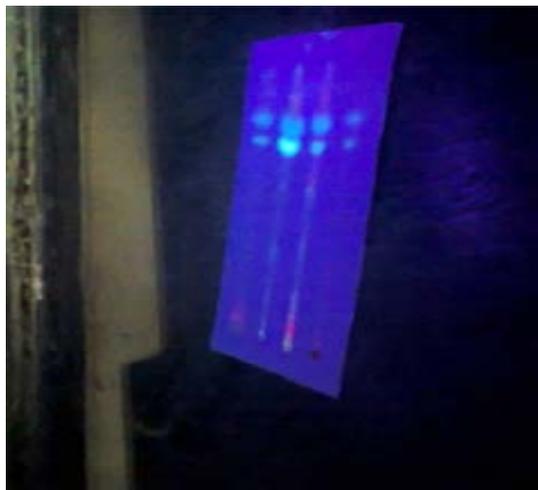
As seen in the bellow plates it can be concluded that the best separation occurs when the mixture of petroleum ether: diethyl ether: glacial acetic acid in a ratio of 80:20:1 respectively was used as a solvent. Almost all extracts contain components of approximate similar R_f values



P. Ether: diethyl ether: glacial acetic acid, 80:20:1



Toluene: ethyl acetate, 93:7



Toluene: ethyl acetate, 70:30

Fig 3: TLC profile of petroleum ether extract

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3.4 GC-MS profile of petroleum ether extract

The GC-MS analysis of PE extract gave seven compounds with 99.08% (E)-cinnamaldehyde with traces of six other components. One of these six components was cyclohexyl-2-phenyl ester of the phthalic acid (0.0784%); three of them (0.3337%) were sesquiterpene derivatives of the naphthalene type along with copaene (0.3273%). A derivative of (E)-cinnamaldehyde named 3-(2-methoxyphenyl) -2- propenal comprised only 0.1766% of the PE extract.

4. Conclusion

From the results obtained in this study, it could be concluded that *C. zeylanicum* possesses remarkable antimicrobial activity which is mainly due to (E)-cinnamaldehyde. The activity is increased progressively when (E)-cinnamaldehyde is separated from other components as they may retard or decrease its activity. According to these findings, it could be said that the petroleum ether extract act as antifungal agent more effectively than being antibacterial agent.

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