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Antibacterial Effect of Cinnamon (*Cinnamomum zeylanicum*) Bark Extract on Different Bacterial Isolates

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ABSTRACT

In this study, the antibacterial activity of Cinnamon (*Cinnamonum zeylanicum*) bark ethanolic extract was investigated on different bacterial isolates (*Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*) of clinical origin. The ethanolic extract of Cinnamon was extracted from the Cinnamon bark (spice) powder using ethanol, and various standard concentrations of the Cinnamon extract were aseptically impregnated onto sterile circular discs. Pure culture of the bacterial isolates was subjected to antimicrobial susceptibility test using the Agar Disc Diffusion method. The findings of this study revealed *E. coli* produced the highest susceptibility to the cinnamon extract; *S. aureus* was intermediately susceptible while *P. aeruginosa* was least susceptible to the highest concentration of the cinnamon extract. Reasonably, the lowest concentration (20 % (v/v)) of Cinnamon extract also had minimal antibacterial action only on *S. aureus* but *E. coli and P. aeruginosa* exhibited resistance to this concentration of Cinnamon bark ethanolic extract. This study portrayed Cinnamon as an antibacterial agent and serves as a pointer for pharmaceutical industries in producing effective antibacterial drugs of plant sources.

INTRODUCTION

Herbal Medicine is the use of plants for medicinal purposes and many of the herbs and spices used by humans to season food also yield useful medicinal compounds [1]. These herbs can be grown from seeds collected from parts of larger trees or from unwanted weeds in nature. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies including opium, aspirin, and quinine. Fabricant *et al.* [2] also related that among about 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic uses and the traditional use of the plants from which they are derived. Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones which have been used worldwide in traditional medicine to treat several diseases and infection [3]. Many studies all over the world have demonstrated that these plants and their extract have multi-antimicrobial properties. While 25 to 50 % of current pharmaceuticals are derived from plants, none is used as antimicrobials [4]. Many conventional drugs originated from plant sources [5]. The use of herbs to treat disease is almost universal among non-industrialized societies [6].

Pharmaceuticals are prohibitively expensive for most of the world's population, half of which live on less than \$2 per day [6], thus the need to exploit the use of herbal medications are

prompted. According to the World Health Organization [7], approximately 25% of modern drugs used in the United States have been derived from plants and 80% of the populations of some Asian and African countries presently use herbal medicine for some aspects of primary healthcare. Among the important medicinal plant is the Cinnamon which is a common spice used by different cultures around the world for several centuries. It is obtained from the inner bark of trees from genus Cinnamomum, a tropical evergreen plant that have two main varieties which includes Cinnamomum zeylanicum (CZ) and (Cinnamomum aromaticum) commonly known as Cinnamon cassia. Cinnamomum zeylanicum (CZ), also known as Ceylon cinnamon or true cinnamon, is indigenous to Sri Lanka and southern parts of India [8]. Interestingly, the number of people utilizing alternate and herbal therapy is growing exponentially [5]. Herbal medicines are normally very popular in developing countries with a long tradition in the use of medicinal plants and in some developed countries where appropriate guidelines for registration of such medicines exist [9]. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants [10].

In addition to its culinary uses, in native Ayurvedic medicine cinnamon is considered a remedy for respiratory, digestive and gynecological ailments. Almost every part of the cinnamon tree including the leaves, bark, flowers, fruits and roots has some medicinal or culinary use [11]. Some of the antimicrobial components that have been identified in spices and herbs are eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, allicin from garlic, cinnamic aldehyde from cinnamon, allyl isothiocyanate from mustard, [12]. Spices are mainly used for flavoring and they also have certain medicinal properties and are used in pharmaceutical, perfumery, cosmetics and several other industries [13].

Antibiotic therapy in recent years has faced difficulties due to the increasing rapid emergence of multidrug resistance among bacteria and fungi causing several life-threatening infections and this in turn making the future management of infectious diseases uncertain. The study of antibacterial activity of Cinnamon may identify and portray Cinnamon as a potential antibacterial medicine and serves as pointer for pharmaceutical industries in producing most effective drugs from plant source. This study aimed at assessing the antibacterial effect of Cinnamon (*Cinnamomum zeylanicum*) bark extract against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Materials

Materials used in the study include the dried Cinnamon bark, Ethanol, Filter paper, Paper puncher, Distilled water, Gram staining reagents, Kovac's reagent, Hydrogen peroxide, 1% Sulfuric acid, 1.175% barium chloride, and blood serum.

Clinical Bacteria Isolates

The clinical bacteria isolates used in the study include: *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* These were collected from Federal Teaching Hospital Gombe and subjected to proper identification and confirmation.

Collection and Preparation of Plant Material

Dried Cinnamon bark was purchased at a spice unit in a supermarket in Gombe metropolis. The Cinnamon bark was grinded into powder using laboratory blender and then sieved with mesh to obtain very fine powder then stored at room temperature in a dry and sterile container.

Extraction

Extraction was done according to Parekh and Chanda, [14] in which 25 gram each of the Cinnamon powder was weighed and soaked in 250 mL of ethanol in conical flasks for two weeks at room temperature with regular shaking. It was then filtered, solvent evaporated and kept at 4 °C before sensitivity testing. The same procedure was carried out for the aqueous extraction using distilled water as the solvent.

Identification and Confirmation of Bacteria Isolates

Clinical bacteria isolates obtained from Federal Teaching Hospital Gombe were subjected to standard laboratory procedure such as Sub-culturing, purification of isolates, Gram staining, microscopy and various biochemical tests for proper identification and confirmation of Isolates. Such biochemical tests include:

Sub-culturing and Purification of Bacteria Isolates

Stock cultures of *E. coli, S. aureus* and *P. aeruginosa* were collected from Federal Teaching Hospital Gombe and subcultured on nutrient agar. Colonies of fresh cultures of the different test organisms from the overnight sub-culture plates were picked with sterile inoculating loop and pure cultures of the test were obtained by streak plating method. Distinct colony were picked from the pure culture plate and sub-cultured on agar slants to obtain stock culture for each of the test organism.

Gram Staining

This was employed in the identification of Gram-positive and Gram-negative microorganisms. A smear of the bacterial isolate is made on a clean slide and stained with gram staining reagents. Their gram reaction and morphology were observed under the microscope using x100 objective lens.

Biochemical test

Confirmatory biochemical test that includes Catalase test, Coagulase test, Indole test, Citrate Utilization test, Motility test, Urease test, and Kligler's Iron Agar test were carried out to confirm the isolates.

Standardization of Inoculum

The identified and confirmed pure bacteria isolates from an overnight were emulsified into peptone water in a test tube using a sterile wire loop then incubated for 24 h at 37 °C. From the overnight, 1 mL was dispensed in a sterile test tube, diluted with distilled water by direct dilution then compared with 0.5 McFarland's standard [15].

Preparation of Stock and Standard Concentrations of Cinnamon Extract

The crude and undiluted extract of Cinnamon assumed 100 % (v/v) was used as the stock concentration. From the stock, several concentrations of 20, 40, 60 and 80 % (v/v) were prepared by diluting with distilled water.

Preparation of Sensitivity Disc

Disc of 6 mm diameter were punched from Whitman's No. 1 filter paper using a paper puncher. Batches of discs were placed into Bijou bottles and sterilized by autoclaving at 121 °C for 15 minutes. The discs were impregnated with the prepared standard concentration of the ethanolic extract of *C. zeylanicum* bark 20, 40, 60, 80 and 100 % (v/v) concentration by separately dispensing the prepared concentrations into the bijou bottles containing the sterilized discs. This was also done for 20, 40, 60, 80 and 100 % (v/v) concentration for the aqueous extract to obtain the sensitivity discs for both the ethanolic and aqueous extracts of *C. zeylanicum* bark 16].

Antimicrobial Susceptibility Test

Agar Disc diffusion method was used for the antibacterial assay. Sterile swab sticks were used to swab the standardized inocula of the test organisms onto the surface of prepared Mueller Hinton agar. And then sterile forceps were used to carefully place the sensitivity discs impregnated with different concentrations of ethanolic and aqueous extracts of *C. zeylanicum* on the surface of the culture media plates. All inoculated plates were inverted and incubated at 37 °C for 24 h, then diameter zones of inhibition in each plate was measured using a meter rule [15].

RESULTS

Table 1 shows the biochemical test results confirming the clinical bacteria isolates thus relating the microscopy and biochemical properties of the test organisms.

Table 1: Microscopy and biochemical characteristics of clinical bacteria isolates

	Biochemical Tests											
Morph.	Gram Rxn	Cat	Coa	Cit	Ind	Mot	Ure	KIA				
								Gas	H ₂ S	Slp	Butt	Organisms
srs	-	+	-	-	+	+	-	+		Y	Y	E. coli
сс	+	+	+	-	-	-	-	-	-	Y	Y	S. aureus
srs	-		-	+		+	-	+	-	R	Y	P. aeruginosa

The results of the antibacterial susceptibility/resistance of the clinical bacteria isolates to various concentrations of ethanolic and aqueous extracts of cinnamon bark shows that the clinical bacteria isolates (E. coli, S. aureus and P. aeruginosa) were susceptible to the ethanolic and aqueous extracts. The ethanolic extract showed comparatively higher antimicrobial activity than the aqueous extract as seen in the Figures 1, 2 and 3. The highest zone of inhibition shown by the ethanolic and aqueous extracts of cinnamon tested on E. coli was 26.5 mm and 13 mm at 100 % (v/v) concentration respectively while the lowest was 9 mm and 7.5 mm at 40 % (v/v) also for ethanol and aqueous extract respectively (Fig. 1). The highest and the lowest zones of inhibition produced by ethanolic and aqueous extracts tested against S. aureus were 20 mm and 11 mm at 100 % (v/v) while lowest produced was 9 mm and 8 mm by 20 % (v/v) and 40 % (v/v) concentration respectively (Fig. 2). The highest and the lowest zones of inhibition produced by ethanolic and aqueous extracts tested against P. aeruginosa were 16 mm and 13 mm at 100 % (v/v) and 9 mm and 7 mm at 40 % (v/v) concentration respectively (Fig. 3).

It is clearly observed that ethanolic extract had higher antimicrobial activity than the aqueous, and the varying sizes of the zones of inhibition were proportional to the concentration of extract. This means that the zones of inhibition increase as the concentration of the extracts increases. It was observed that *E. coli* and *P. aeruginosa* conferred resistance to 20 % (v/v) of both aqueous and ethanolic extracts of cinnamon with no zones of inhibition for both the extracts (**Fig. 1** and **3**). However, *S. aureus* was susceptible to the 20 % (v/v) ethanolic extract with 9 mm zone of inhibition but also conferred resistance to 20 % (v/v) of the aqueous extract (**Fig. 2**).



Fig. Antimicrobial susceptibility/resistance of *E. coli* to various concentrations of ethanolic and aqueous extracts of *Cinnamomum zeylanicum* bark.



Fig. 2. The antimicrobial susceptibility/resistance of *S. aureus* to various concentrations of ethanolic and aqueous extracts of *Cinnamomum zeylanicum* bark.



Fig. 3. The antimicrobial susceptibility/resistance of *P. aeruginosa* to various concentrations of ethanolic and aqueous extracts of *Cinnamomum zeylanicum* bark.

DISCUSSION

In this study antibacterial activity of ethanolic and aqueous extracts of Cinnamomum zeylanicum bark was tested on E. coli, S. aureus and P. aeruginosa. Zones of inhibition were observed for both ethanolic and aqueous extracts of C. zevlanicum bark thus indicating the plant has reasonable antibacterial activity or potency against these tested organisms (E. coli, S. aureus and P. *aeruginosa*) of clinical origin. The antibacterial activity of C. zeylanicum denoted in this study may be attributed to the presence of phyto ingredients; this is in accordance with the work of Uma et al. [17] who reported that the antimicrobial activity of C. zeylanicum bark is mainly due to the presence of phytochemicals such as cinnamaldehyde, ethyl cinnamate, ßcaryophyllene, eugenol, and tarpenes in C. zevlanicum. However, in this study screening for phytochemicals of C. zevlanicum was not achieved but several studies has demonstrated the presence of active antimicrobial components in C. zeylanicum [17,18].

It may be worthy to state that these active components of C. zevlanicum were importantly responsible for its antimicrobial effects as earlier inferred by Uma et al. [17]. Research have shown that cinnamaldehyde exhibits its antimicrobial activity due to its lipophilicity of terpenoids and phenyl propanoids which can penetrate the membrane and reach the inner part of the cell and impair bacterial enzyme system which leads to killing of the microorganism [19]. Subsequently, as reported by Asha et al. [20], these phytochemical compounds are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen bonding to membrane proteins and destructing the membranes, electron transport system and cell wall. The antimicrobial activity of essential oils obtained from cinnamon is bactericidal - these literatures further buttress claims attributing phyto ingredient to the antibacterial activities of Cinnamon (21).

This study shows ethanolic *Cinnamomum zeylanicum* bark extract had considerably higher antibacterial activity than the aqueous extract as seen in Figures provided; this is in accordance with the work of Mukhtar and Ghori, [22] who concluded that the antimicrobial component of *Cinnamomum zeylanicum* bark is more soluble in ethanol which is an organic solvent than in water resulting in the release of active antimicrobial component. It is most evident from the Fig. 1, 2 and 3 that the zones of inhibition produced by *C. zeylanicum* for both ethanolic and aqueous extracts is proportional to the concentration of the extract; this importantly implies that the zones of inhibition obtained increases as the concentration of *C. zeylanicum* extract increases for both ethanolic and aqueous extract. This phenomenon is expected and is in agreement with the study carried out by Matan *et al.* [23].

CONCLUSION

Conclusively, the antibacterial activity of cinnamon was studied, and cinnamon was found to possess antibacterial activity on *E. coli, S. aureus* and *P. aeruginosa* of clinical origin, this can be attributed to its active phyto ingredients. The highest zones of inhibition observed proves the effectiveness of the use of cinnamon for traditional medication and confirms that it can be used as antimicrobial agent especially at increased concentrations. The findings of this study confirmed the medicinal potential of cinnamon bark, as cinnamon was found to possess reasonably high antibacterial activity on *E. coli, S. aureus* and *P. aeruginosa* from clinical source. Therefore, Cinnamon can be regarded an important antimicrobial which should be exploited industrially for the production of modern drugs for the treatment of a wide range of microbial infections, and to combat antimicrobial resistance. The result of this study confirms the medicinal potential of cinnamon bark as an important antimicrobial and can be used for the production of modern drugs for the treatment of a wide range of microbial infections. It is therefore recommended that (i) Further research on the active compounds responsible for the antimicrobial activity of cinnamon should be carried out, (ii) Studies on bacteriostatic and bactericidal effect of cinnamon on other important clinical pathogens should be carried out and (iii) Extensive studies and detailed investigation on the antifungal activities of cinnamon should be undertaken.

REFERENCES

- Tapsell LC, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS, et al. Health benefits of herbs and spices: the past, the present, the future. Med J Aust. 2006;185(4):1–24.
- Fabricant DS, Farnsworth NR. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ Health Perspect. 2001;109:69–75.
- Sadi NS, Sunusi U, Kamal RM, Ibrahim S, Muhammad MN. Effect of Hibiscus sabdariffa Calyx Extract on Some Biochemical and Histological parameters of Wistar Rats with Carbon Tetrachloride Induced Liver Damage. Sokoto J Med Lab Sci. 2018;3(4):46–57.
- Ababutain IM. Antimicrobial Activity of Ethanolic Extracts From Some Medicinal Plant. Aust J Basic Appl Sci. 2011;5(11):678–83.
- Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian Pac J Cancer Prev [Internet]. 2003;4(4):281–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14728584
- DaSilva EJ, Baydoun E, Badran A. Biotechnology and the developing world. Electron J Biotechnol. 2002;5(1):0717–3458.
- World Health Organization. Monographs on Selected Medicinal Plants Volume 2. WHO monographs on selected medicinal plants, Volume 2. 2002. p. 55–65.
- Paranagama PA, Wimalasena S, Jayatilake GS, Jayawardena AL, Senanayake UM, Mubarak AM. A comparison of essential oil constituents of bark, leaf, root and fruit of cinnamon (*Cinnamomum zeylanicum* blum) grown in Sri Lanka. J Natl Sci Found Sri Lanka. 2001;29(3–4):147–53.
- Calixto JB. Efficacy, Safety, Quality control and Marketing, and Regulatory guidelines for Herbal Medicines (Phytotherapeutic agents). Brazilian J Med Biol Res. 2000;33:179–89.
- Carubba A, Scalenghe R. Scent of Mare Nostrum: Medicinal and Aromatic Plants (MAPs) in Mediterranean soils. J Sci Food Agric. 2012;92(6):1150–1170.
- Wang SY, Chen PF, Chang ST. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresour Technol. 2005;96:813–8.
- Skrinjar M, Nemet N. Antimicrobial effects of spices and herbs essential oils. Acta Period Technol. 2010;220(40):195–209.
- Vangalapati M, Sree Satya N, Surya Prakash D V., Avanigadda S. A review on pharmacological activities and clinical effects of Cinnamon species. Res J Pharm Biol Chem Sci. 2012;3(1):653–63.
- Parekh J, Chanda S. In-vitro antibacterial Activity of Crude Methanolic Extract of Woodfordia Fruticosa Kurz Flower (Lythacease). Brazilian J Microbiol. 2007;38:2–10.
- 15. Cheesbourgh M. District laboratory practice in tropical countries. In: part 2 Thatford press. 2005. p. 64–70.
- Ibrahim S, Bello AS, Sunusi U, Lere MY, Umar FS, Egbong UD, et al. Phytochemical screening and anti-microbial activities of the leaf, stem bark and root extracts of Combretum sokodense. Bayero J Pure Appl Sci. 2017;10(2):11–5.
- Prabhakar UB, Rajendran K, Lakshmi SY. Studies on GC/MS Spectroscopic analysis of some bioactive Antimicrobial compounds from *Cinnamomum zeylanicum*. J Med Plants. 2009;8(31):125–31.
- Pandey S, Pandey R, Singh. R. Phytochemical screening of selected medicinal plant Cinnamon Zeylanicum bark extract. Int J Sci Res. 2014;4(6):1–6.
- Rajesh P, Rajesh KW, Latha S, Selvamani P. Phytochemical and pharmacological profile of plants belonging to Strychnos genus; a review. Bioact Phytochem Perspect Mod Med. 2014;1:275–327.

- Asha S, Nithisha K, Bharath KR, Ravi K V. Deciphering the antimicrobial potential of *Cinnamomum zeylanicum* bark. Int J Pharmacol Technol Res. 2014;6(11):1226–35.
- Senhaji O, Faid M, Kalalou I. Inactivation of E. coli O157:H7 by Essential oils from *Cinnamomum zeylanicum*. Brazilian J Infect Dis. 2007;11(2):233–9.
- Mukhtar S, Ghori I. Antibacterial activity of aqueous and ethanolic extracts of Garlic, Cinnamon and Turmeric against *Escherichia coli* ATCC 25922 and Bacillus subtilis DSM 3256. Int J Appl Biol Pharm Technol [Internet]. 2012;3(2):131–6. Available from: www.ijabpt.com
- Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. Int J Food Microbiol. 2006;107:180–185.