European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 26-01-2020; Accepted: 27-02-2020; Published: 28-02-2020
www.biosciencejournals.com
Volume 8; Issue 1; 2020; Page No. 26-29

In vitro antimicrobial efficacy of *cinnamon zeylancium* against clinically isolated pathogenic microorganism and comparison with antibiotic streptomycin

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Abstract
Herbs and spices are used not only as antioxidants and flavoring agents, but also for their antimicrobial activity against degradation induced by food borne pathogens and food spoilage bacteria. In present time, plants are considered the greatest source to obtain new antimicrobials. They produce secondary metabolites, phytochemicals, which protect the plants against pathogens. The aim of this study was to assess the antimicrobial efficacy of *Cinnamon zeylanicum* in the various solvents that are ethanol, methanol, acetone, distilled water, ethyl acetate, benzene, hexane, chloroform, petroleum ether, DMSO extracts of cinnamon bark were done for activity against medically important microorganism that are *Escherichia coli, Staphylococcus aureus, Pseudomonas sp., Bacillus sp., Salmonella typhi, E. faecalis.* The in vitro antimicrobial activity assay was performed by agar well diffusion method and disc diffusion method. *Cinnamon zeylanicum* showed antimicrobial activity in all solvent extracts but extracts of methanol, acetone and ethyl acetate has effect that is more bacteriostatic than other extracts. The minimum inhibitory concentration of methanol extract was found 10µl. Acetone, methanol and ethyl acetate extracts of cinnamon bark showed significant amount of antimicrobial activity with comparison to antibiotic streptomycin. In present scenario, when bacteria are growing resistance against commonly used antibiotics, plant derived antimicrobial against can be used in therapeutics.

Keywords: antimicrobial efficacy, streptomycin, MIC & clinically isolated pathogens

Introduction
Drug discovery from plants has been done from ancient time and plants are explored to get important drugs. The use of antibiotics has revolutionized the treatment of various bacterial infections with ease but their indiscriminate and unregulated use has led to an alarming increase in antibiotic resistance among microorganism. In modern medicine, around a quarter of the drugs prescribed to patients are derived from medicinal plants. The tribal and rural people of various parts of India are highly depending on medicinal plants therapy for meeting their health care needs, India has been known to be rich repository of medicinal plants. The Indian forest have large number of medicinal and aromatic plants, which are largely used as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH system in INDIA. Ayurveda, Unani, Siddha and Folk medicines are the major system of indigenous medicines. In India Ayurveda and Unani medicine are commonly used in treatment of various diseases. The chemotherapy requires continuous search for new drugs that can counter the challenge posed by resistant strains. The investigation of certain naturally occurring indigenous plants for their antimicrobial properties can provide useful results (Arora and Kaur, 1999). In many countries, there is little regulation of traditional medicine, but the WHO coordinates a network to encourage safe and rational usage. A pharmacological study has now accepted the value of medicinal plants as prospective source of bioactive compounds. The World Health Organization (WHO) estimates that 80% of the world`s population depends mainly on traditional medicines for their health care. The use of plant-based medicine including herbal and natural health products with benefits, is increasing in developing countries. Many medicinal plants have proved to with success aid in varied ailments resulting in mass screening for his or her therapeutic elements. Today the seek for natural compounds wealthy in antimicrobial properties square measure escalating thanks to their medicinal importance in dominant several diseases. The rapid emergence of multiple drug resistant strains of pathogens to current antimicrobial agents has generated an urgent intensive search for new antibiotics from medicinal plants (Chopra *et.al*., 1996). The emergence of resistant bacterial and fungal strains due to overuse of antibiotics is a cause of worldwide concern. The use of plant extracts and phytochemicals with known antimicrobial properties may have great significance in therapeutic treatments (Aneja *et al*., 2009). Herbs and spices are used not only as antioxidants and flavoring agents, but also for their antimicrobial activity against degradation induced by foodborne pathogens and food spoilage bacteria. Recent time, a large demand had risen for preservative free cosmetics and antimicrobial herbal extracts, to aimed for decreasing the risk of allergies connected to synthetic preservatives. Antimicrobial of plant origin have enormous therapeutic potential. They are effective in the treatments

while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The useful medicative effects of plant materials generally result from the combos of secondary merchandise gift within the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body. After many research, shows that plants are rich sources of different classes of antimicrobial substances acting as defense systems to protect them against biotic (living) and a biotic (non-living) stresses. Medicinal plants have provided man an outsized style of potent drug to alleviate or eradicate infections and laid low with diseases in spite of advancement in artificial medicine, some of the plant-derived still retained their importance and relevance. The use of plant-based drugs all over world is increasing (Bhat and Jacobs, 1995). Cinnamon (generally called Dalchini) is a piece obtain from the inner bark of several tree species from the genus *Cinnamomum*, which contains more than 300 evergreen aromatic trees and shrubs. The tree attains a height of 10-15M and leaves are ovate-oblong in shape and 7-18cm long. The fruit could be a purple, 1cm fruit containing one seed. *Cinnamomum zeylanicum* is a tree of family Lauraceae native to Shri Lanka (Ceylon), the neighboring Malabar Coast of India and Myanmar (Burma) and also cultivated in South. Ground cinnamon is composed of around 11% water, 81% carbohydrates, 4% protein, 1% fat. Moreover, the rich sources of calcium iron and vitamin k. Cinnamon constituents include some 80 aromatic compounds, including eugenol found in the oil from leaves or bark of cinnamon trees (Kazemi and Mokhtariniya, 2016). Cinnamon bark is common ingredient in several product like dentifrice, mouthwash, perfume, soap, lipstick, chew gum, cough sweetener, nasal sprays and cola drinks, And for flavorer. The cinnamon bark harvested from the young branches is primarily employed in cookery are literally items of rolled outer bark.

Material and Methods

Collection of Plant Material: *Cinnamon zeylanicum* barks were collected from Dehradun Uttrakhand India.

Solvent Extraction: Barks of Cinnamon were collected and then cut into small pieces and pulverized to powder using a mortar and pestle. And the powder was preserved in air tight container. Solvent extraction was done by Soxhlet extraction method. Cinnamon bark powder was mixed with ethanol and distilled water was placed in a thimble, which is placed in thimble chamber of the Soxhlet apparatus. Extraction solvents were heated in the bottom flask, vaporizes into a sample thimble, condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. The extracts were prepared by taking 5 g of cinnamon barks powder which were mixed in 50 ml of solvents (methanol, ethyl acetate, acetone, benzene, distilled water, ethanol, hexane, petroleum ether, chloroform and DMSO). The extract were filtered with Whatman filter paper in a beaker, and weight of empty beaker was measured, then it was kept at water bath at 56ºC and 65ºC till it gets evaporated and again weight was measured.

Procurement of Microorganisms: The clinically isolated pathogens (*Escherichia coli, Staphylococcus aureus, Pseudomonas sp., Bacillus sp., Salmonella typhi, and E. faecalis*) were obtained from Shri Mahant Indiresh Hospital Dehradun, and were maintained in Nutrient agar plate.

Antibacterial Activity Assay: Antibacterial activity of the different extract was determined by well diffusion method and disc diffusion method (Bauer *et al.*, 1966). In well diffusion method, 50 ml of medicinal plant extract was filled in the wells on nutrient agar plate and in another well antibiotic streptomycin was filled with the help of micro pipette on agar plates spread with respective microorganism. In disc diffusion method, The 20ml of sterilized Nutrient agar media was poured into sterile Petri plate, after solidification, 50 µl of fresh culture of clinically isolated pathogens were swabbed on respective plates. The discs soaked in solvent extracts were kept over the agar plates using sterile forceps. The plates were incubated for 24 hours at 37ºC. After incubation the diameter of inhibitory zone formed around each discs were measured (mm) recorded.

Antimicrobial efficacy of antibiotics: The antimicrobial efficacy of cinnamon extracts on medically pathogenic microorganisms was compared with the antibiotics. Sterile Nutrient agar plates were prepared and microorganisms were spread over agar plates. The fresh solution of antibiotic streptomycin was prepared (10mg in 1ml). Antibiotic was filled in wells of well diffusion plates and antibiotic discs were placed on surface of disc diffusion plates. After incubation of 24 hours in 37ºC, the zone of inhibition was measured (mm) and recorded.

Minimum Inhibitory Concentration (MIC) Determination: It was done for determining bacterial growth inhibition by growth kinetics assay, a few test tubes, each containing 5 ml of sterile broth medium were taken. Then in each tube 200µl of individual bacterial cultures of *Staphylococcus aureus, Bacillus, E. coli, S. typhi, Pseudomonas and E. faecalis* were added. Agar plates were prepared earlier and then 200ul from that test culture was added to the agar plates & spread by spreader. Wells of uniform diameter were made on solidified agar plate using a sterile borer. One well in each Petri plate contained the pure solvent as control & in other three wells different concentration 100ul, 50ul, 20ul and 10ul of Cinnamon extract in ethanol was added. Then all the Petri plates were kept in incubator shaker at 37 degree Celsius overnight and next day the zone of inhibition on different concentrations were measured. Similar experiment was done using Ethyl acetate extract of barks.

Results and Discussion

Antimicrobial Efficacy of Solvents extracts: Ten solvents were used to extract plant Cinnamon. Ten solvent extracts showed different antimicrobial potential against clinical pathogens. Here are the results of 10 solvents extracts of antimicrobial effect test-

1. **Acetone extract:** The antimicrobial activity of acetone extracts of cinnamon was assayed. *S. aureus* and *bacillus* have showed maximum zone of inhibition (37mm). The collective analysis of antimicrobial activity of acetone extract indicated that all six pathogenic microorganism have better impact range from 21 to 37mm. whereas, in disc diffusion method, *E. coli* showed maximum zone of inhibition (24mm), other bacteria have 0 to 24mm.
2. **Methanol extract:** Methanol extract of Cinnamon plant in well diffusion method showed maximum antimicrobial activity against *Bacilus* in which it

showed 37mm of zone of inhibition and other bacteria had zone of inhibition range from 23 to 38mm. Whereas by using disc diffusion method, *S. typhi* showed maximum zone of inhibition (25mm), Other bacteria showed zone of inhibition range from 0 to 25mm of cinnamon methanol extract.

3. **Ethyl acetate extract:** Cinnamon extract in ethyl acetate in well diffusion method showed maximum zone of inhibition (40mm) against *S. typhi*. Where others pathogen also have good affect from 12 to 40mm. In disc diffusion method, *S. typhi* (30mm) showed maximum zone of inhibition and remaining have range from 12 to 30mm.
4. **Benzene extract:** In agar well diffusion method, benzene extract of cinnamon against *Bacillus* showed maximum zone of inhibition (42mm) and other bacteria showed better impact from zero to 42mm. In disc diffusion method, *S. typhi* showed maximum zone of inhibition (27mm) and other bacteria showed zone of inbibition from 11 to 27mm.
5. **Distilled water extract:** The observation antimicrobial activity, aqueous extracts of cinnamon on pathogen using agar well diffusion method *S. typhi* (21mm) showed maximum zone of inhibition and other bacteria showed better zone. Using disc diffusion method, *Pseudomonas* (21mm) have better zone of inhibition and other pathogen have good impact.
6. **Ethanol extract:** The antimicrobial assay of ethanol extract of cinnamon on pathogenic microorganism using agar well diffusion method, *Bacillus* (37mm) has showed maximum zone of inhibition whereas other pathogen also showed good impact. In disc diffusion method *Bacillus* (27mm) showed maximum zone of inhibition, and remaining pathogen plates also showed zone of inhibition.
7. **Petroleum ether extract:** In the antimicrobial activity of petroleum ether extracts on clinically isolated pathogens by using agar well diffusion method, *E. faecalis*(30mm) has showed maximum zone of inhibition whereas other pathogen have showed zone of inhibition. In disc diffusion method *S. typhi* (20mm) showed maximum zone of inhibition and other pathogens showed zone of inhibition from 12 to 20mm.
8. **Chloroform extract:** In agar well diffusion method of chloroform cinnamon extract on pathogens, *Bacillus* (31mm) showed maximum zone of inhibition whereas other pathogen also showed good impact. In disc diffusion method *E. faecalis*, *Pseudomonas*, *E. coli* showed equal zone of inhibition (17mm) while other pathogen showed good impact from 12 to 17mm.
9. **Hexane extract:** Hexane extract of cinnamon on pathogenic microorganisms assayed for antimicrobial activity by using agar well diffusion method *E. faecalis* (18mm) has showed maximum zone of inhibition while other pathogen have good impact from 15 to 18mm. Using disc diffusion method *Pseudomonas* (17mm) showed maximum zone of inhibition and other shoed from 7 to 17mm.
10. **DMSO extract:** In antimicrobial assay of cinnamon DMSO extract using well diffusion method on pathogenic microorganism, *S. typhi* (23mm) has showed maximum zone of inhibition whereas other bacteria showed good impact from 9 to 23mm. In disc diffusion method, *Pseudomonas* (17mm) showed maximum zone of inhibition and other pathogen showed in between from 9 to 17mm.

Table 1: Antimicrobial activity of Cinnamon extracts on clinically isolated pathogen by Agar well diffusion method.

S. N.	Solvent extracts	Zone of Inbition against Bacteria (in mm)					
		Pseudomonas	*S. typhi*	*S. aureus*	*Bacillus*	*E. faecalis*	*E. coli*
1	Acetone	26	31	37	37	32	21
2	Methanol	25	30	34	38	23	23
3	Benzene	25	5	27	42	28	20
4	Ethyl acetate	27	35	25	20	15	12
5	Distilled water	14	21	18	18	20	20
6	Ethanol	19	29	19	37	28	_
7	Petroleum ether	18	19	16	25	30	20
8	Chloroform	15	30	20	31	20	30
9	Hexane	-	16	17	16	18	15
10	DMSO	16	23	20	15	9	15

Table 2: Antimicrobial activity assay of Cinnamon extract on clinically isolated pathogen by Disc diffusion method.

S. N.	Solvent extracts	Zone of Inbition against Bacteria (in mm)					
		Pseudomonas	*S. typhi*	*S. aureus*	*Bacillus*	*E. faecelis*	*E. coli*
1	Acetone	9	-	13	_	_	24
2	Methanol	15	25	19	22	_	17
3	Benzene	12	27	11	33	22	17
4	Ethyl acetate	20	30	22	20	12	19
5	Distilled water	21	17	_	14	10	15
6	Ethanol	19	_	15	27	19	_
7	Petroleum ether	12	20	12	18	18	10
8	Chloroform	17	13	14	12	17	17
9	Hexane	17	12	10	13	10	7
10	DMSO	17	9	10	15	16	10

High Activity Showing Solvents: Acetone, methanol and ethyl acetate showed higher activity against clinically isolated pathogens. Their antimicrobial activities were compared with the antimicrobial activity of the antibiotic streptomycin.

1. **Acetone extract antimicrobial activity comparison with antibiotic:** The antimicrobial activity of acetone extract of Cinnamon plant was compared with antibiotic streptomycin by well diffusion method. The comparison is shown in Figure 1.

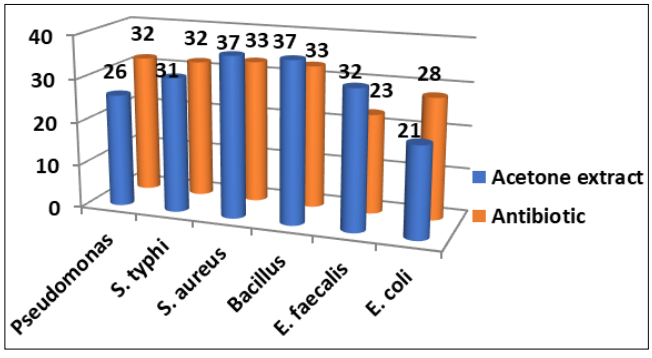

Fig 1: Comparison of antimicrobial activity of Acetone extract of Cinnamon plant and Streptomycin antibiotic using Agar well diffusion method.

2. **Comparison of antimicrobial activity of methanol extract of Cinnamon plant with antibiotic:** The activity of methanol extract of Cinnamon plant was compared with streptomycin extract using well diffusion method. Comparison is shown in figure 2.

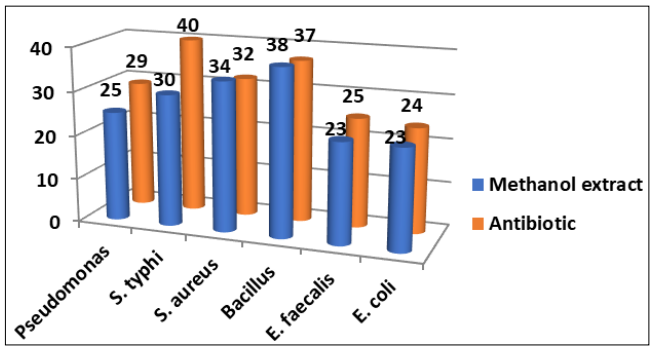

Fig 2: Comparison of antimicrobial activity of Methanol extract of Cinnamon plant and Streptomycin antibiotic using Agar well diffusion method**.**

3. **Comparison of antimicrobial activity of Ethyl acetate extract of Cinnamon plant with antibiotic:** Comparison of antimicrobial activity of Ethyl acetate extract and streptomycin antibiotic is shown in Figure 3.

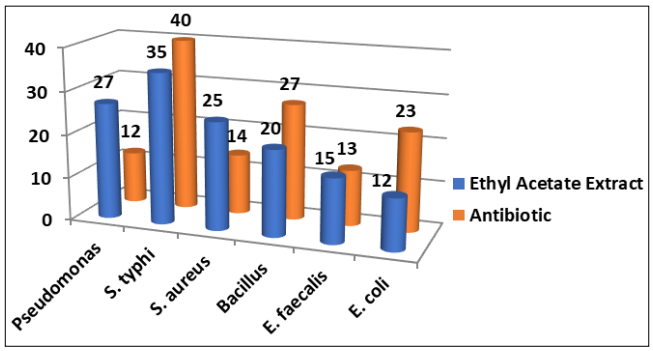

Fig 3: Comparison of antimicrobial activity of Ethyl acetate extract of Cinnamon and streptomycin antibiotic using Agar well diffusion method.

MIC (Minimum Inhibitory Concentration) Determination: Minimum inhibitory concentration of Cinnamon plant extract on nutrient agar media for each microorganism was determined by modified agar well diffusion method. Methanol extract was used to determine minimum inhibitory concentration. Methanol extract of Cinnamon plant showed antimicrobial activity at lowest concentration 10µl.

Table 3: Minimum inhibitory concentration (MIC) determination of methanol extract of Cinnamon plant.

Methanol Cinnamon extract (10µl)	Zone of Inbition (in mm) against Bacteria					
	Pseudomonas	S.typhi	S.aureus	Bacillus	E.faecelis	E. coli
	15	12	8	10	6	10

Conclusion

On comparison of antimicrobial activities of all ten solvent extracts against clinically isolated pathogenic microorganism, it was observed that by using agar well diffusion method Methanol, Acetone and Ethyl acetate cinnamon extracts have much higher antimicrobial activity then others. In disc diffusion method of all ten solvent extract showed fine amount of antimicrobial activity against clinically isolated pathogens. Results obtained from this study, indicate that, traditionally used Cinnamon plant extract has much similar or stronger antimicrobial activity then antibiotics. This can be helpful for the further studies or screening of Cinnamon plant that will find new antibacterial, antifungal compounds against other pathogenic bacteria, fungus and clinical pathogens.

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