World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Antimicrobial activity of selected medicinal plants cinnamon and clove

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Received: 21-06-2020 / Revised Accepted: 25-07-2020 / Published: 31-07-2020

ABSTRACT

The present research work attempts to evaluate the antimicrobial activity of Ethanol and Methanol extracts of two traditional medicinal plants viz. *Cinnamomum zeylanicum, Syzygium aromaticum.* Since ethanol is the best solvent for dissolving the antimicrobial compound present as active phytoconstituents in plants, which extracted with ethanol. Each extract was tested for its antimicrobial activity against Eight most prevalent pathogenic microorganism *Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus, Aspergillus niger* The preliminary antimicrobial activity testing was carried out by using agar well diffusion method and minimum inhibitory concentrations of the extract was then determined by using the micro broth dilution assay. All these plant extracts exhibited antimicrobial activity against tested microbes the ethanol & methanol extract of *Cinnamomum zeylanicum* showed the highest antimicrobial activity against *Micrococcus luteus* & *Staphylococcus aureus* with the zone of inhibition 16 mm.

Key Words: *Cinnamomum zeylanicum, Syzygium aromaticum,* Antimicrobial Activity, *Bacillus subtilis, Escherichia coli, Eneterobacter aerogenes, Micrococcus luteus, Aspergillus niger*

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How to Cite this Article: Dhara Harsoda, Roshani Chaudhari, Nikita Chavada, Dhaval Prajapati. Antimicrobial activity of selected medicinal plants cinnamon and clove. World J Pharm Sci 2020; 8(8): 74-81.

INTRODUCTION

Medicinal plants are nothing but a blessing to us as they are the ample bio-resource of drugs for Traditional medicines, food supplements, modern medicines, pharmaceutical intermediates, and chemical units for synthetic drugs. (Moon, 2018), the utilization of plant and its items features a long history that started with folk medication and through a long time has been consolidated into traditional and allopathic medicine (Dubey *et al.*, 2011).

Pharmacological ponders have acknowledged the esteem of restorative plants as potential source of bioactive compounds (Aneja *et al.*, 2009). A restorative plant may be a plant that's utilized with the purposeful of keeping up wellbeing, to be managed for a particular condition, or both, whether in present-day medication or conventional pharmaceutical. With the progression in Science and Innovation, remarkable progress has been made within the field of medication with the discoveries of numerous common and engineered drugs. *Cinnamomum cassia* (family Lauraceae), commonly called cinnamon, is an evergreen plant. The extracts of its bark contain numerous active chemicals like essential oils including cinnamyl aldehyde, carbohydrates, bodily fluid, and tannin. These compounds have anti-inflammatory, anti-microbial, anti-tumor and anti-diabetic properties. (Rasool *et al.,* 2019) Impaired apoptosis plays critical roles in the initiation and progression of cancer. (Sadegh *et al.,* 2019)

Cinnamon is utilized within the smell and essence industries due to its scent, which can be incorporated into diverse assortments of foodstuffs, fragrances, and medicinal products, (Huang *et al.,* 2007) Clove and its derivatives have a definite potential to be used as specific anti-plaque and anti-inflammatory agents for the treatment of periodontal disease (Pulikottil & Nath 2015). , as WHO (World Health Organization) evaluated that 80 percent of individuals around the world depend on herbal solutions for a few aspects of their essential health care needs. Agreeing to WHO, around 21,000 plant species have the potential for being utilized as therapeutic plants (NIHFW, India) Plant resources have remained an integral part of human society throughout history. After fulfilling the primary needs like food and shelter, man has sought for a suitable remedy among plants for curing various diseases (WHO, Traditional Medicine: Growing Needs and Potentials, 2002.) About 16% of herbal medicines; there was limited in vitro or in vivo evidence for roughly half the medicines; there was only phytochemical evidence for around 20%; 0.5% were allergenic or toxic, and some 12% had never been studied scientifically (Cravotto, 2010). Clove owes its value to the aromatic essential oil, obtained from the steam distillation of powdered clove buds or leaves. Predominant bioactive phytochemicals present is eugenol [2-methoxy-4-(2-propenyl) phenol]. (Singletary, 2014)

Clove possesses antidiabetic, anti-inflammatory, antithrombotic, anesthetic, pain-relieving, and insect-repellent properties (Parle & Khanna, 2011).)The main ingredients of clove are eugenol (50–87%), eugenol acetate, tannins, thymol, and β-caryophyllene (Ghorab & Massr, 2003).

MATERIAL AND METHOD

Bark, flower sample and extract preparation

Collection of Bark (*Cinnamomum zeylanicum*): It had a pale yellowish color, sweet aroma and had a sweet finish in the after taste. Flowers (*Syzygium aromaticum*) strong and sweet with a bitter, astringent flavor as well from the native market of Rajkot. Alcoholic extracts were prepared by different compounds. The extraction of methanol solvent (cinnamon and cloves) and extraction of ethanol (cinnamon and cloves) are put on a shaker at 120rpm and 37±2°C for 48 hrs. (Pandey & Singh, 2011).

Extraction of cinnamon and cloves into Ethanol

The 20 gm of cinnamon and cloves powder are taken added into the 50% of ethanol solvent for the preparation of 50 % ethanol, take the 50 ml ethanol and 50 ml of D/W. (Sun *et al.,*2015)

Extraction of cinnamon and cloves into Methanol

The 20 gm of cinnamon and cloves powder are taken and added into 100 ml of methanol solvent for extraction. (Larson *et al.,* 2016)

DMSO (dimethyl sulfoxide)

DMSO is used as a standard for these works. DMSO is a by-product of papermaking. It arises from a substance found in wood. DMSO is easily absorbed by the skin. It's sometimes used to increase the body's absorption of other medications. (Restiana *et al.,* 2018)

Well diffusion method

Nutrient agar and molted Were prepare and sterilized at 121°C temperature and 15lbs pressure for 15 min. After autoclaving, the 15 ml of nutrient agar is poured into sterile Petri plate and solidified it. The 12 ml of malted agar is cool at approximate 40°C.After 200 µl of the test, organism sample is added and Mix it well. The molted agar is poured on the surface of a nutrient agar plate and solidified. Make the well with the help of cup borer on the nutrient agar with test organism plate. The

diameter of the borer is 8 mm. After making the well, load the 100 µl sample into the well. The loaded plate is incubated at 37°C for 24-48 hrs. After the incubation zone was observed.(Jahangirian *et al.,* 2013, Sen & Batra 2012).

Paper disc method

Nutrient agar was prepared and sterilized at 121°C temperature and 15lbs pressure for 15 min. After autoclaving the media and cool it at approximate 40°C. The test organism of 100 µl is added into the nutrient agar, mix it well. The 20 ml of nutrient agar with test organism is poured into sterile Petri plate and solidified. Cut the paper of 6 mm diameter. The paper is deeped into the sample and put on the plate with the help of forceps. (Valgas *et al.,* 2007).

After the solvents were filled into centrifuge tube and the centrifuge at 8000 rpm and 37°C for 20 min. After centrifuge, the supernatant and pellets were separated. The supernatant was taken and filtered with the help of Wattman filter paper no.1.The pre-weight and post weights were recorded. The filtrates were evaporated in a water bath. After the evaporation, the jelly forms were observed. Add different concentrations of DMSO in a jelly form of solvent. The 5% DMSO is added into the extracted of cinnamon in both ethanol and methanol solvent, 6% DMSO is added into the extraction of methanol of cloves and 10% DMSO is added into the extraction of ethanol of cloves. These solutions are used as a sample (Jain *et al.,* 2015).

Microorganisms for Test

Gram-positive Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Micrococcus luteus, S. aureus and Gram-negative Salmonella typhi, Escherichia coli, Enterobacter aerogenes pathogenic bacteria were used for the inhibition zone method, Plants Extract were used for evaluation of an antimicrobial activity. The bacterial stock cultures were incubated in nutrient agar for 24hrs at 370C

Determination of antimicrobial activity

Antimicrobial activity was determined by the Agar well diffusion method by (Jahangirian *et.,al*, 2013, Sen & Batra, 2012) and Agar diffusion disc (Valgas *et.,al* 2007)

RESULTS AND DISCUSSION

The anti-bacterial activity of the *Cinnamomum zeylanicum* and *Syzygium aromaticum* bark and flower extracts were studied against seven bacterial species and on Fungal Species is summarized in Table 1 and Table-2. The results revealed that the selected extracts showed antibacterial activity with varying values. The zone of inhibition above 7 mm in diameter was taken as a positive result.

TABLE-1 EXTRACTION OF CINNAMON PLANT

Organism	*Well diffusion Method*		*Disc Diffusion method*	
	Ethanol(mm)	*Methanol(mm)*	*Ethanol(mm)*	*Methanol(mm)*
Staphylococcus aureus	12	16	10	16
Salmonella typhi	9	11	7	12
Bacillus cereus	13	15	11	10
Bacillus subtilis	9	10	6	7
Escherichia coli	8	11	7	0.9
Eneterobacter aerogenes	11	12	8	10
Micrococcus luteus	10	16	9	9
Aspergillus niger	3	2	0	0

TABLE-2 EXTRACTION OF CLOVES PLANT

Organism	*Well diffusion Method*		*Disc Diffusion method*	
	Ethanol(mm)	*Methanol(mm)*	*Ethanol(mm)*	*Methanol (mm)*
Staphylococcus aureus	9	11	10	10
Salmonella typhi	7	5	4	3
Bacillus cereus	14	12	11	11
Bacillus subtilis	8	9	7	8
Escherichia coli	10	4	10	9
Eneterobacter aerogenes	11	9	8	7
Micrococcus luteus	10	7	9	11
Aspergillus niger	2	1	0	0

TABLE-3 Results of MIC

Organism	*Cinnamomum zeylanicum*	*Syzygium aromaticum*
Staphylococcus aureus	1/128	1/128
Salmonella typhi	1/128	1/64
Bacillus cereus	1/128	1/128
Bacillus subtilis	1/256	1/128
Escherichia coli	1/128	1/128
Eneterobacter aerogenes	1/128	1/ 128
Micrococcus leteus	1/256	1/64
Aspergillus niger	1/512	1/512

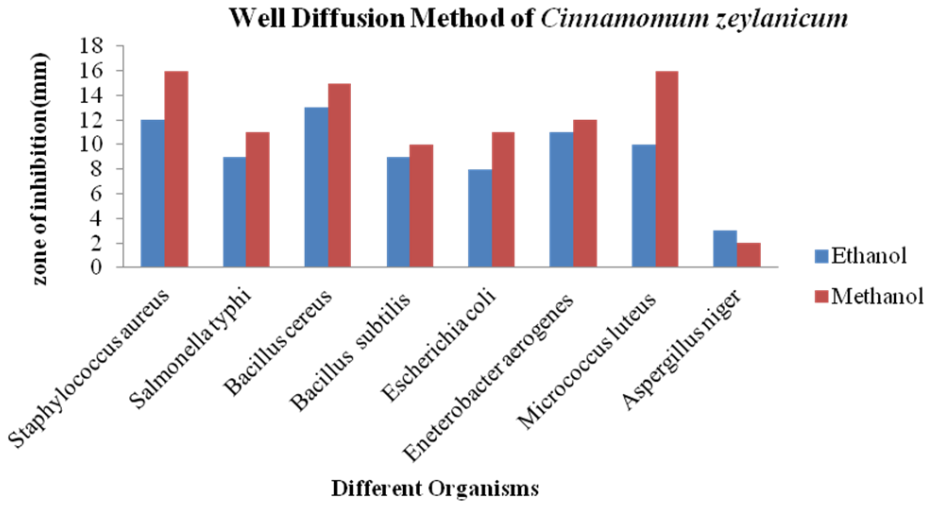

Figure 1: Effect of Methanol and Ethanol Extract on Different Organisms

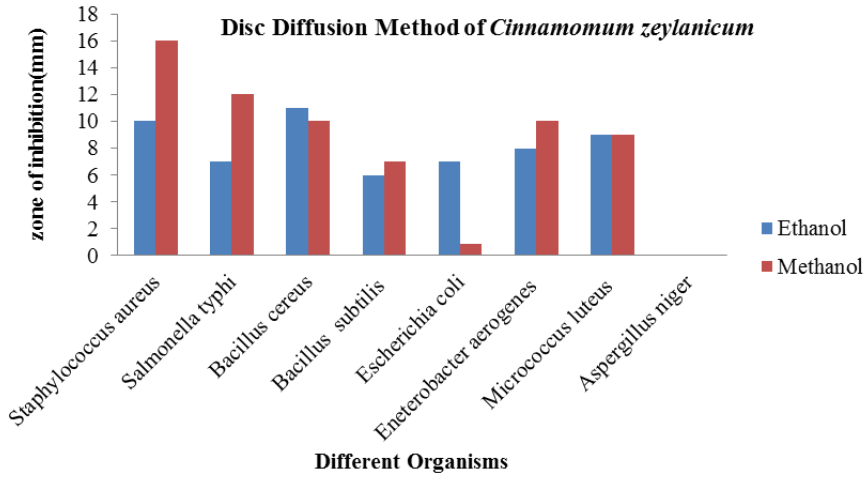

Figure 2: Effect of Methanol and Ethanol Extract on Different Organisms

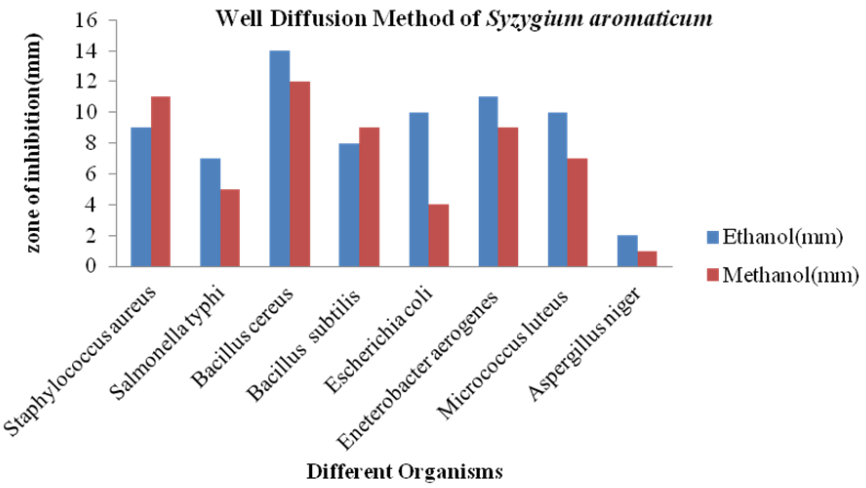

Figure 3: Effect of Methanol and Ethanol Extract on Different Organisms

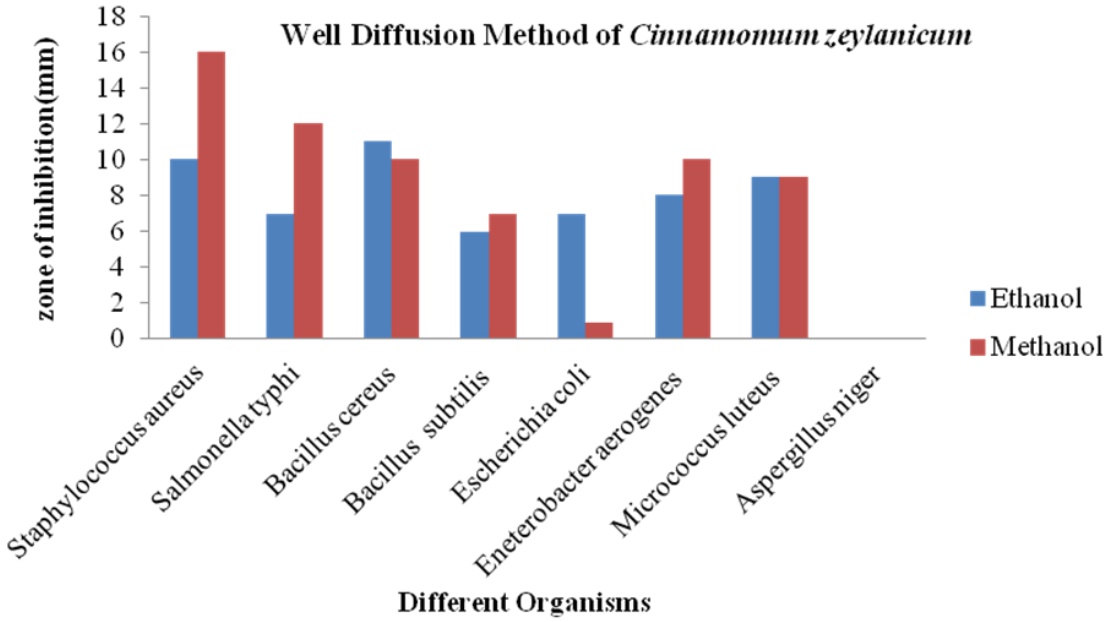

Figure 4: Effect of Methanol and Ethanol Extract on Different Organisms

Two different methods were used for *extract Cinnamomum zeylanicum,& Syzygium aromaticumin* their medicinal product part which of extraction flower and bark of plants, The Microbial activity of this extract was checked against selective Gram-Negative and Gram-Positive organisms. There extract was used for Antimicrobial activity towards organisms. Diverse zone appears concerning both Methods Cup borer and paper Disk Method.with *organisms Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus, Aspergillus niger.*

For *Cinnamomum zeylanicum*
Well Diffusion Method
Ethanol extraction: *Bacillus cereus* (13 mm) appear the highest zone as respect to other organisms like *Staphylococcus aureus* (12 mm), *Enterobacter aerogenes* (11 mm), *Micrococcus luteus* (10 mm), *Salmonella typhi & Bacillus subtilis* (9 mm), *Escherichia coli* (8 mm) respectively was observed.

Methanol extraction: From Methanol extraction Staphylococcus aureus & Micrococcus luteus (16 mm) give the highest zone as respective to, *Bacillus cereus* (15 mm), *Enterobacter aerogenes* (12 mm), *Salmonella typhi & Escherichia coli* (11 mm), *Bacillus subtilis (*10 mm) and *Aspergillus niger* below of it observed.

Disk Diffusion Method
Ethanol extraction: In this Disk Diffusion method, extracted by ethanol *Bacillus cereus(11mm), explore the highest zone as compare to other organisms like Staphylococcus aureus (10mm), Micrococcus luteus (9 mm), Eneterobacter aerogenes(9 mm), Escherichia coli (7 mm)* respectively other are below than 7 mm are considered as Negative.

Methanol extraction: *In methanol extraction of **Cinnamomum zeylanicum** Staphylococcus aureus (16 mm) appear the highest zone, Salmonella typhi (12 mm), Bacillus cereus,* & *Eneterobacter aerogenes (10 mm),Bacillus cereus&Micrococcus luteus (9 mm), Bacillus subtilis (7 mm)* and other are observe below.

For *Syzygium Aromaticum*
Well Diffusion Method
Ethanol extraction by well Diffusion method *Bacillus cereus(14 mm) represent the highest zone as compare to other like, Eneterobacter aerogenes (11 mm), Micrococcus luteus & Escherichia coli (10 mm) Bacillus subtilis (8 mm) and Salmonella typhi (7 mm)* observed

Methanol extraction: *Bacillus cereus (12 mm), Staphylococcus aureus* (11mm), *Bacillus subtilis* (9 mm*) and Micrococcus luteus (*7 cm*)* others express less effective are found.

Disk Diffusion Method
Ethanol extraction: This *Bacillus cereus (12 mm), Staphylococcus aureus* (11mm), *Escherichia coli (10 mm), Micrococcus luteus (7* cm*)* and other are lower them 7 mm zone of diameter.

Methanol extraction: *Bacillus cereus & Micrococcus luteus (11 mm), Staphylococcus aureus* (10mm), *Escherichia coli (9 mm), Bacillus subtilis* (8 mm*)* other less effective than above organisms

The MIC Results
The MIC results of the *Cinnamomum zeylanicum* extracts indicated that antibacterial activities at lower concentrations (1/128) on *Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Escherichia coli were Micrococcus leteu*s (1/256) *Aspergillus niger* (1/512) was reported. Similarly, in the case of *Syzygium aromaticum, Salmonella typhi & Micrococcus leteus* (1/64), *Staphylococcus* aureus, *Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes* (1/128) and *Aspergillus niger* (1/512) were Reported.

DISCUSSION

The exploration of antimicrobials from natural resources has received much and put in to identify compounds that can act as a suitable antimicrobials agent to replace synthetic ones. Phytochemicals derivative from plant products serves as an exemplar to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds have significant healing applications against human pathogens including bacteria, fungi or viruses. The study has been conducted with the extracts of plants, screening antimicrobial activity (Kelmanson *et., al,* 2000), (Ahmad and Beg ,2001). The medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food Supplements.

In the present study, different extracts of *Cinnamomum zeylanicum, & Syzygium aromaticumin* was evaluated for exploration of their antimicrobial activity against certain Gram-negative and Gram-positive bacteria, fungus which was stared as human pathogenic microorganism. The defenselessness of each plant extract was tested by the serial microdilution method (MIC) and the agar well diffusion method was determined.

Our primary investigation showed that all Ethanol, Methanol & extracts of Cinnamomum zeylanicum, & Syzygium aromaticumin were active against the locally isolated human pathogens like Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus, Aspergillus niger.

CONCLUSION

In conclusion, the present investigation Cinnamomum zeylanicum, & Syzygium aromaticumin contain potential antimicrobial

components that may be of great use for the development in pharmaceutical industries. The ethanol, methanol, extracts of Cinnamomum zeylanicum, & Syzygium aromaticumin possess significant inhibitory effect against tested pathogens. A primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antifungal and antibacterial compounds against .pathogens and the resistant bacterial and fungal pathogens where the common plant parts which will open the possibilities of finding fallow clinically successful antibacterial compounds against the resistant bacteria pathogens. Hence broad investigate is required to discover out the instrument of activity of other compounds for different diseases.

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