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ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF *CINNAMOMUM ZEYLANICUM* AND *ARTEMISIA ABSINTHIUM* OIL AGAINSTTHE FOOD BORNE MICROBES

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ABSTRACT

The aim of the present study was to assess the Antimicrobial activity and phytochemical analysis of *Cinnamomum zeylanicum* and *Artemisia abisinthium* oil against some of the food borne microbes. The antimicrobial activity was performed by agar well diffusion assay for both bacteria and fungi. The oil of *Cinnamomum zeylanicum* shows higher inhibitory activity against the clinical pathogens *Escherichia coli*, *Bacillus* sp., *Salmonella* sp., *Pseudomonas* sp., *Staphylococcus aureus* and among the fungi *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Fusarium* sp., when compared to *Artemisia abisinthium* oil. The antimicrobial activity of oil was found to be very effective with a lowest concentration (MIC) of 0.5% against *Escherichia coli*, *Bacillus* sp., *Salmonella* sp., *Pseudomonas* sp., *Staphylococcus aureus.* The phytochemical shows the presence of Saponins, Phenols, Flavonoids, Tannins, Amino acids, Carbohydrates. Therefore, this study shows that *Cinnamomum zeylanicum* oil is a more potent antimicrobial agent than *Artemisia abisinthium* oil and that has the potential to be used as Food bio preservatives.

KEYWORDS: Antimicrobial, Phytochemicals, Pathogens, Bio preservatives.

INTRODUCTION

Species are one of the most commonly used natural antimicrobial agents in food and have

been traditionally for thousands of year by many culture for preserving foods and as food[1], additives has resin in recent years, consumer additives has risen in recent years, consumer interest in the use of natural products as alternative food preservatives has increased.[2]

The bark of various cinnamon species is one of the most important and popular species used worldwide not only for cooking but also in traditionally and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus. *Cinnamomum zeylanicum* is one of the oldest herbal medicines.[3,4] In addition, there are studies have shown that cinnamon essential oils might be effective against oral infections.[5]

Bacteria resistant to antibiotics derived infections are nowadays considered as one of the largest problems faced by medicines and food industries, due to requirement for more difficult, cumbersome, and costly processes to diagnosis and treat the related severe infection. For many years, plants and plant derived metabolites have served as a the starting point for the discovery and development of new antimicrobial agents.[6]

Artemisia absinthium -Wormwood (Asteraceae), is an herbaceous plant. This species has numerous (about20) synonymous Latin names.[7,8,9] Among the English names, the most popular is "Wermet" meaning "keeping a clear mind". *Artemisia absinthium* is a shrub-like perennial plant growing to a height of 80 cm. In the habitats, sharp smell. *Artemisia absinthium* leaves essential oil secreting hairs / glandular trichomes and covering T-hairs that have a protective function they protect the plant against high temperatures and prolonged drought.[10]

An in-depth literature survey revealed that different *Artemisia* species have been traditionally used as antidiabetic, anthelmintic, tonic, antimalarial, and antiulcer agents as well as for the treatment of bronchitis, wound inflammation and tuberculosis. Many researchers have also explored the antioxidant, antipyretic, analgesic, antimicrobial, cytotoxic, and antifungal activities of different *Artemisia* species.[11]

MATERIALS AND METHODS

Collection of oil samples

The oil sample of *Cinnamomum zeylanicum* and *Artemisia absinthium* was purchased from the supermarket in Erode.

Collection of Clinical Pathogens

The Clinical Pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella* sp., *Bacillus* sp., *Pseudomonas* sp., cultures were collected from the Bioline Laboratory, R S Puram, Coimbatore.

(i) ANTIBACTERIAL ANALYSIS[12] Preparation of broth cultures

All the five clinical pathogens (*Escherichia coli, Staphylococcus aureus, Salmonella sp., Bacillus* sp., *Pseudomonas* sp.,) was cultured in Nutrient broth and grown overnight at 37˚c for 24 hours, it was used in antibacterial analysis.

MEDIA PREPARATION

Mueller Hinton agar (MHA) Composition (grams/ L)

HM infusion B form- 300.00

Acicase	-17.50
Starch	-1.50
Agar	-17.00
Final p^H	-7.3±0.1

About 38 gram of MHA (Muller Hinton Agar) was dissolved in1000 ml of distilled water and then autoclaved for 15 minutes at 121˚c. Once the medium was about 45˚-50˚c, it was poured into sterile petri dishes. Then it was allowed to set completely.

Antibacterial activity

Antibacterial activity was performed by Agar well diffusion assay. The 20µl of 24 hours broth culture was swabbed on MHA plates with a sterile cotton swabs and allow the plates for 2-3 minutes. The well was punctured with a well cutter, then 30µl of oil samples (*Cinnamomum zeylanicum* and *Artemisia absinthium*) was loaded in a well. Oxacillin was used as a positive control and DMSO was used as a negative control. The plates was incubated at 37˚c for 24 hours. After incubation, the diameter of zone of inhibition (mm) was measured and noted.

Determination of Minimum Inhibitory Concentration (MIC) of oil samples

The MIC was defined as the lowest concentration that completely inhibited the growth of microbes for 24 hours. The MIC for the oil was determined by the agar well diffusion method. *Cinnamomum zeylanicum* and *Artemisia absinthium* oil was then diluted in sterile DMSO to achieve a decreasing concentration 0.5% to 2.5% was prepared. A 50µl volume of each dilution was added aseptically into the wells in Mueller Hinton agar (MHA) plates that

had been inoculated with standardized inoculums of the test bacteria. The agar plates were incubated at 37°C for 24 hours. Sodium propionate serves as positive control. The lowest concentration of *Cinnamomum zeylanicum* and *Artemisia absinthium* oil showing a clear zone of inhibition was considered as the MIC.

ii) ANTIFUNGAL ACTIVITY[13]Collection of fungal specimens

The fungal cultures such as *Aspergillus niger, Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., were collect from Bioline laboratory, Coimbatore.

MEDIA PREPARATION

SD broth composition (Gram /L)

Dextrose (Glucose)	20.000	Peptone, Special	10.000
Final PH (at 25°C)	5.6 ± 0.2		

About 30 grams of SD broth was dissolved in 1000ml of distilled water and then autoclaved for 15 minutes at 121°C. Once the medium was above 45°C to 50°C, it was poured into sterile petri dishes.

Preparation of fungal broth cultures

All the four fungal specimen was cultured in Sabouraud's Dextrose Broth growing at 28°C for 3 days, it was used in antifungal analysis.

Antifungal Activity

Antifungal activity was performed by agar well diffusion method. The 15µl of broth culture was swabbed on SDA plates with sterile cotton swabs and allow the plates for 2-3minutes. The well was punctured with a well cutter then 50µl of oil samples was loaded on a wells. Sodium Propionate was used as a positive control. DMSO was used as a negative control. The plates were incubated at room temperature at 28°C for 3 days. After incubation the diameter of zone of inhibition (mm) was measured and recorded.

(iii) PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical analysis of the oil sample as follows, TEST FOR ALKALOIDS (Hager's test)

About 2 ml of oil sample was mixed with few ml of dilute hydrochloric (Hcl) acid and added with few drops of Hager's reagent (Aqueous solution of Picric acid). A yellow precipitate indicates the presence of Alkaloids.

TEST FOR PROTEINS

About 2ml of oil samples, 1 ml of 40% sodium hydroxide solutions and two drops of 1% copper sulphate solution was added. Violet colour shows the presence of protein.

TEST FOR CARBOHYDRATES

About 1-2 ml of oil samples, equal amount of fehlings solution A and B were added and on heating if brick red colour occurs, it shows the presence of carbhohydrates.

TEST FOR STERIODS

About 2 ml of oil sample was mixed with 5 ml of chloroform, 2 ml of acetic anhydride and 1ml of concentrated sulfuric acid (H_2So_4) and the colour changes was observed. Reddish brown colour indicates the presence of Steroids.

TEST FOR FLAVONOIDS (Alkaline reagent test)

About 2ml of oil sample, few drops of NaOH solution was added, a yellow colour solution was formed. Then add 1ml of diluted hydrochloric (Hcl) acid which turns yellow colour solution into a colorless solution, which indicates the presence of Flavonoids.

TEST FOR TANNINS (Braymer's test)

To a small amount of oil sample add 2ml of ferric chloride ($FeCl_3$) and the colour change was recorded. The formation of green grey / dark blue colour indicates the presence of Tannins.

TEST FOR SAPONINS (Foam test)

The oil sample and the distilled water was mixed as same volume and the mixture was shaken vigorously. The formation of a foam layer indicates the presence of Saponins.

TEST FOR AMINO ACIDS

Two drops of ninhydrin solutions was added to the oil sample in order to show the presence of amino acids in oil samples.

TEST FOR PHENOLS (Ferric chloride test)

About 2 ml of oil sample, 1 ml of ferric chloride ($FeCl_3$) solution was added. Deep blue black colour indicates the presence of Phenols.

TEST FOR QUINONES

About 1 ml of oil samples, 1 ml of concentrated sulfuric acid was added. Appearance of red colour shows the presence of quinones.

RESULTS AND DISCUSSION

Antibacterial activity of *Cinnamomum zeylanicum* and *Artemisia absinthium* oil

From the results obtained, the cinnamon oil inhibits the majority of some food borne microbes. *Bacillus* sp., *Staphylococcus aureus, E. coli, Salmonella* sp., was found to be most sensitive to *Cinnamomum zeylanicum* oil. *Pseudomonas* was found to be resistant to *Cinnamomum zeylanicum, Artemisia absinthium* oil. (Table 1&2)

Table 1: Antibacterial Activity OF *Cinnamomum zeylanicum* OIL.

S. No.	ORGANISM	ZONE OF INHIBITION (mm)		
		***Cinnamomum zeylanicum* oil**	**Positive control (Oxacillin)**	**Negative control(DMSO)**
1.	*Bacillus* sp.,	32	-	-
2.	*Staphylococcus aureus*	34	13	-
3.	*Salmonella* sp.,	30	26	-
4.	*Escherichia coli*	25	22	-
5.	*Pseudomonas* sp.,	26	16	-

Table 2: Antibacterial Activity of *Artemisia absinthium* OIL.

S. No.	ORGANISM	ZONE OF INHIBITION (mm)		
		***Artemisia absinthium* oil**	**Positive control (Oxacillin)**	**Negative control (DMSO)**
1.	*Bacillus* sp.,	17	-	-
2.	*Staphylococcus aureus*	22	13	-
3.	*Salmonella* sp.,	13	26	-
4.	*Escherichia coli*	11	22	-
5.	*Pseudomonas* sp.,	-	-	-

Minimum inhibitory concentration of *Cinnamomum zeylanicum* and *Artemisia absinthium* oil

MIC value of *Cinnamomum zeylanicum, Artemisia absinthium* oil concentration between 0.5-2.5%. While the *Cinnamomum zeylanicum* oil inhibits the growth of both groups of (Gram positive and Gram Negative) bacteria. (Table 3&4).

Table 3: Minimum Inhibitory Concentration of *Cinnamomum zeylanicum* OIL.

S.No	Organism	Zone of inhibition (mm)				
		0.5%	1%	1.5%	2%	2.5%
1	*Bacillus* sp.,	14	15	15	20	13
2	*Staphylococcus aureus*	12	12	11	14	16
3	*Salmonella* sp.,	10	13	12	14	14
4	*Escherichia coli*	10	16	15	20	20
5	*Pseudomonas* sp.,	13	15	16	17	15

Table 4: Minimum Inhibitory Concentration of *Artemisia Absinthium* Oil.

S.No	Organism	Zone of inhibition (mm)				
		0.5%	1%	1.5%	2%	2.5%
1	*Bacillus* sp.,	9	13	18	17	8
2	*Staphylococcus aureus*	12	14	11	11	12
3	*Salmonella sp.*,	10	9	10	11	10
4	*Escherichia coli*	14	17	18	19	21
5	*Pseudomonas* sp.,	11	12	10	12	14

Antifungal activity of *cinnamomum zeylanicum* and *Artemisia abisinthium* oil

The antifungal study has shown that *cinnamomum zeylanicum* oil sensitive to *Aspergillus flavus, Fusarium* sp.,. *Aspergillus niger, Penicillium* sp., was found o be resistant to *cinnamomum zeylanicum, Artemisia absinthium* oil.(Table 5&6).

Antifungalactivity of *cinnamomum zeylanicum and artemisia absinthium* Oil

Table 5: *Cinnamomum zeylanicum* oil.

S. No.	Organism	ZONE OF INHIBITION (mm)		
		Cinnamomum zeylanicum oil	Positive control (Sodium propionate)	Negative control (DMSO)
1.	*Aspergillus niger*	13	18	-
2.	*Aspergillus flavus*	23	11	-
3.	*Penicillium* sp.,	8	-	-
4.	*Fusarium* sp.,	13	30	-

Table 6: *Artemisia absinthium* oil.

S. No.	Organism	Zone of inhibition (MM)		
		Artemisia absinthium oil	Positive control (Sodium propionate)	Negative control (DMSO)
1.	*Aspergillus niger*	-	18	-
2.	*Aspergillus flavus*	9	11	-
3.	*Penicillium* sp.,	-	-	-
4.	*Fusarium* sp.,	18	30	-

Phytochemical Analysis of Oil Samples

Majorly, all the phytochemicals like Saponins, Phenols, Flavonoids, Tannins, Aminoacids

and Carbohydrates were present in *Cinnamomum zeylanicum* and *Artemisia abisinthium* oil. The phytochemicals like Proteins and Steriods were absent in *Cinnamomum zeylanicum* and *Artemisia absinthium* oil. The Quinones were absent in *Cinnamomum zeylanicum* oil and present in *Artemisia absinthium* oil.

Table 7: Phytochemical Analysis Of *Cinnamomum zeylanicum AndArtemisia absinthium* OIL.

S. No.	Phytochemicals	*Cinnamomumzeylanicum* Oil	*Artemisia abisinthium* Oil
1.	Steroids	-	-
2.	Saponins	+	+
3.	Phenols	+	+
4.	Flavonoids	+	+
5.	Tannins	+	+
6.	Protein	-	-
7.	Amino acids	+	+
8.	Carbhohydrates	+	+
9.	Quinones	-	+
PRESENT (+) ABSENT (-)			

SUMMARY AND CONCLUSION

The *Cinnamomum zeylanicum* and *Artemisia absinthium* oil was collected from Erode local market. The oil samples were subjected to analyze the antimicrobial activity and the presence of phytochemicals. The antibacterial activity of oil samples was performed by agar well diffusion method against the food borne pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas* sp., *Salmonella* sp., Which was collect from laboratory.

The cinnamon oil shows the higher Antimicrobial activity compared to *Artemisia absinthium* oil. *Artemisia absinthium* shows inhibitory activity against some food born microbes. Hence it represents an alternative source of natural antimicrobial substances for use infood systems to prevent the growth of food borne bacteria and extend the shelf-life of the processed food.

Minimum inhibitory concentration can be determined lower MIC values indicates that less drug is required for inhibitory growth of the organisms; therefore, drugs with lower MIC scores are more effective antimicrobial agents.

The phytochemical analysis demonstrated the presence of metabolites.ie, Phytochemicals in *Cinnamomum zeylanicum* and *Artemisia absinthium* oil has antioxidant property.

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