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# Antimicrobial Activity of Cinnamonextracts against Foodborne Pathogens *E.Coli, S.Tyhimurium and S.Aureus & L.Monocytogens*

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***Abstract:*** *Solvents extracts of Cinnamon (Cinnamomum cassia) were analyzed for determination of antibacterial activity against four food borne pathogens: (4strains), two gram negative, Escherichia coli, Salmonella typhi, and two gram positive Staphylococcus aureus & listeria monocytogens .Screening of cinnamon extracts showed antibacterial activity against the test organisms. Cinnamon extracts showed antibacterial activity against all test bacteria with zone inhibition ranged from 10mm-35mm. MIC values for cinnamon is ranges from 23.04mg/kg to 64.44mg/kg.*
***Keyword:*** *Antibacterial Activity, Cinnamon extracts, Food Borne Pathogens and Spoilage Bacteria.*

## I. Introduction

The term spices refer to aromatic or pungent vegetable substances used for flavoring foods and have several commercial uses according to (ISO). Since ancient times people used spices for preventing food deterioration and pathogenic diseases. Spices have become today as an integral part of our daily diet and many of the spices are widely used to flavor food and beverages, for food preservations, medicinal preparations, cosmetics, perfumery, bakery goods and various other products. Even today spices are used as an ingredient in drug preparations in Unani, Homeopathy and Ayurveda systems of medicine. Phytochemical investigations of the aerial parts of the plants have tartaric acid, acetic acid citric acid, succinic acid, gums, pectin, sugars, tannins alkaloids, flavonoids, glycosides and sesquiterpenes Although, the primary purpose of spices is to impart flavor and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices have also been exploited. The antimicrobial activity of is documented an alarming interest continues to the present.

The growing concern about food safety has recently led to the development of natural antimicrobials to Control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural Antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for Preserving foods and as food additives to enhance aroma and flavor (1).The antimicrobial properties of some spices and their components have been documented (2, 3, 4, and 5). Studies done previously confirm that garlic, onion, cinnamon, cloves, thyme, sage, and other spicesinhibit the growth of both Grampositive and Gramnegative food borne pathogens or spoilage bacteria, yeast, and molds (1,6).

The antibacterial activity of spices may differ between strains within the same species. Moreover, the antimicrobial properties of spices may differ depending on the form of spices added, such as fresh, dried, or extracted form and also differ depending on the harvesting seasonsand between geographical sources. However, there is evidence that the essential oils of spices are more strongly antibacterial than is accounted for by the additive effect of their major antimicrobial components; minor components to play a significant role**.**and cinnamon have been used in foods since antiquity. Major antimicrobial components in and cinnamon have been reported to be and cinnamaldehyde, respectively, which have been given special attention to find their antibacterial activity against food borne pathogens.

The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world [7,8].

Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (9). The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (10). A study on Cinnamomumosmophloeum (C. osmophloeum) indicated that the essential oil from cinnamon leaves contains a high level of Cin. Consequently, C. osmophloeum is also used as an alternative spice for C. cassia (11). One of the major constituents of essential oil extracted from C. zeylanicum named (E)-

cinnamaldehydehas an antityrosinase activity (12) while cinnamaldehyde is the principal compound responsible for this activity (13).

Cinnamon bark contains procyanidins and catechins (14). The components of procyanidinsinclude bothprocyanidin A-type and B-type linkages (15)theseprocyanidins extracted from cinnamon and berries also possess antioxidant activities (16).

## II. Materials And Method

**2.1Collection of plant:**

The Cinnamons were collected from the Western Ghats of Karnataka place called Nittur in shimoga district.

**2.2 Test Microorganisms:**

Food pathogen bacteria were procured from the IMTECH, Chandigarh India, and the cultures were sub cultured and preserved under paraffin oil. *E.coli (MTCC433), S.typhimurium (MTCC98), S.aureus (MTCC96), P.aeruginosa (MTCC2453), L.monocytogens(MTCC1143)*

**2.3 Preparation of extracts:**

Cinnamon was dried in an incubator at 37°C for 3-4 days and grinded into fine powder. Extracts were prepared by using sox let extraction method, petroleum ether, ethyl acetate, ethanol, methanol were used for the spice extracts.Ground cinnamon were added to solvents and extracted for 6-8hrs.Once the extraction completes the solvent was evaporated in Rota vapor and the extract is reconstituted in dimethyl sulfoxide (DMSO)(17).

**2.4 Antimicrobial sensitivity testing:**

The antimicrobial activity of the cinnamon extracts was determined accordingto the method of Bauer et al. Sterile disc were procured from the HimediaIndia , and code of the disc is DD036,were impregnated with 50µl of different concentration of each extracts before being placed on the Inoculated agar plates. The inoculated of the test organisms were prepared by transferring a loopful of cultureinto 9 ml of sterilized Nutrient broth (Himedia) and incubated at 37°Cfor 5 to 6 h(18) .The bacterial culture was compared with

McFarland Standard 1 (MacFarland standard 1 is equal to $3.0x10^8$ cells).100µl of prepared bacterial culture was spreaded on the pre dried Petri plates .Sterile disc were impregnated with 50µl of the extracts. After the inoculum dried, the impregnated discs were placed on the agar plate using forceps dipped in ethanol and flamed, and were gently Pressed down to ensure contact. Plates were kept at $4^0$Cfor 30 to 60 min for better absorption, during this timemicroorganisms will not grow, but absorption of theextracts will take place. Negative controls were prepared using the same solvent without the plant extract. Areference antibiotic, gentamycin, was used as a positive control. The inoculated plates containing the impregnated discs were incubated in an upright positionat $37^0$C overnight for 24 to 48 h. The results wereexpressed as the zone of inhibition around the disc.

**2.5 Determination of the minimum inhibitory concentration (MIC).**

The minimum inhibitory concentrations (MICs) of all the extracts were determined by 96 microtiter well method. Bacterial cells, extracts media were added to the microtiter plate and the initial OD of the plate was recorded by ELISA reader. Simultaneously controls were added and the above procedure repeated. Then the microtiter plates were incubated at $37^0$C for 24hrs, after the incubation the readings were taken and recorded.

Plates were prepared under the aseptic conditions. A sterile 96 well plate was labelled .A volume of 100µl of test material was pipetted into wells as control.

To all other well 50 µl of nutrient broth 50 µl bacterial cells (108 cells) was added +50 µl of the extract added. Then OD was obtained by using ELISA reader (Make Alree AM2100), and then the plates were incubated at $37^0$C for 24hrs. After the incubation again the OD was measured by using ELISA. Antimicrobial activity was determined by subtracting the initial OD by Final OD(19). The above procedure was repeated by diluting the extract.

**2.6 Gas Chromatography - Mass Spectrometry (GC/MS):**

The GC-MS analysis using Trace GC-MSD (Agilent) was performed with a gas chromatograph ultra-Trace equipped with HP-5MS capillary column (30m x 0.25mm; coating thickness 0.25µm) and mass detector. Temperatures of the transfer line and the ionic source were 1500C and 2300C, respectively; scan range, 50-500 amu; 3.9 scans/s. Oven temperature programmed from 1200C and 2800C ramp of 50C /min; injector temperature was 280°C; carrier gas helium at 1 ml/min; injection of 1µl splitless mode .Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series to C –C alkanes, and on computer matching against commercial (NIST-MS) and laboratory-developed library mass spectra built up from pure substances and components of known oils and MS literature data (20)

## III. Results And Discussion

In the present study the antibacterial effect of cinnamon extracts is showed in the bellow. Cinnamon extracts showed antibacterial activity against all test bacteria with zone inhibition ranged from 10mm-35mm.The maximum zone of inhibition was against Gram negative bacteria Salmonella typhimurium (34mm) and E.coli (35mm), than Gram positive bacteria Stphylococcus aureus (40mm) and Listeria monocytogens (34mm). MIC values for cinnamon is ranges from 23.04mg/kg to 64.44mg/kg.Theirwas no antimicrobial activity observed in the diluted extracts. The constituents of Cinnamon extract listed in order of their elution on the HP- 5MS column Cinnamaldehyde (5.64%) Cycloeicosane (10.33) Butyl myristate (6.28) 2-Propenal, 3-phenyl-(5.64) Octatriacontylpentafluoropropionate (2.12) and in the netmug extracts(10.39) Desmethylnomifensin (7.67) Oxazolo(1.72),Thiazole(1.72)

In addition to being used as a spice and flavoring agent, cinnamon is also added to flavor chewing gums due to its mouth refreshing effects and ability to remove bad breath (21). Cinnamon can also improve the health of the colon, thereby reducing the risk of colon cancer (22).

Cinnamon is a coagulant and prevents bleeding (23). Cinnamon also increases the blood circulation in the uterus and advances tissue regeneration (24). This plant plays a vital role as a spice, but its essential oils and other constituents also have important activities, including antimicrobial (25-27), antifungal (28), antioxidant (29-32), and antidiabetic (33-36). Cinnamon has been used as anti-inflammatory, antitermitic(37), nematicidal(38), mosquito larvicidal(39), insecticidal (40), antimycotic, and anticancer agent. Cinnamon has also been traditionally used as tooth powder and to treat toothaches, dental problems, oral microbiota, and bad breath (41, 42).

### 3.1 Tables and Figures

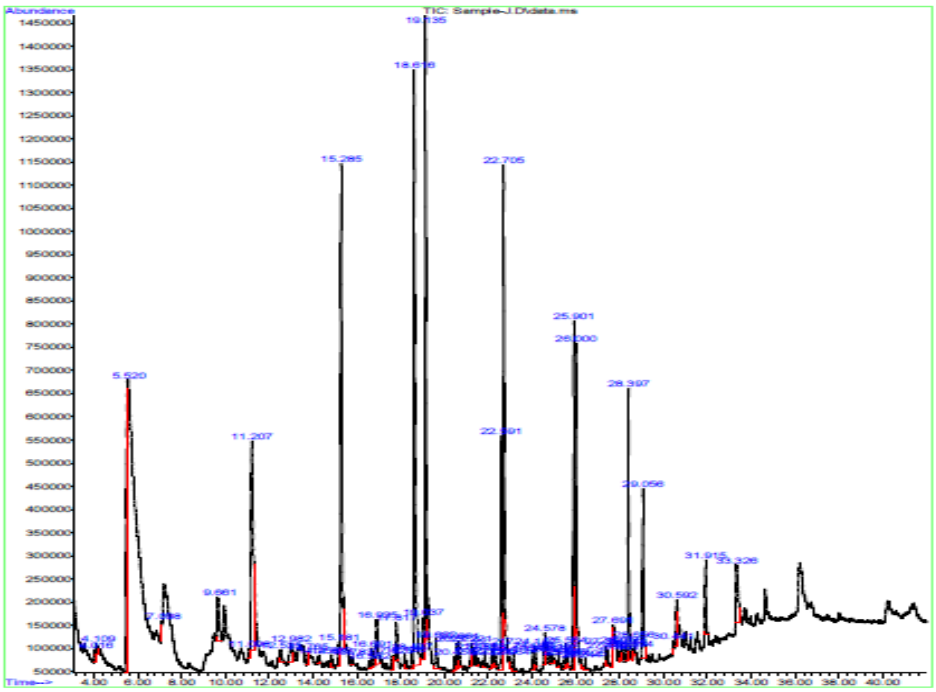
**Antimicrobial activity of Cinnamon extracts**

| Extracts | Bacteria | | | |
|---|---|---|---|---|
| | *E.coli* | *S.typhimurium* | *S.aureus* | *L.monocytogens* |
| Petroleum ether | 30mm | 26mm | 40mm | 30mm |
| Ethyl acetate | 35mm | 34mm | 40mm | 34mm |
| Ethanol | 20mm | 18mm | 10mm | 20mm |
| Methanol | 10mm | 20mm | 10mm | 10mm |

**MIC of Cinnamon extracts**

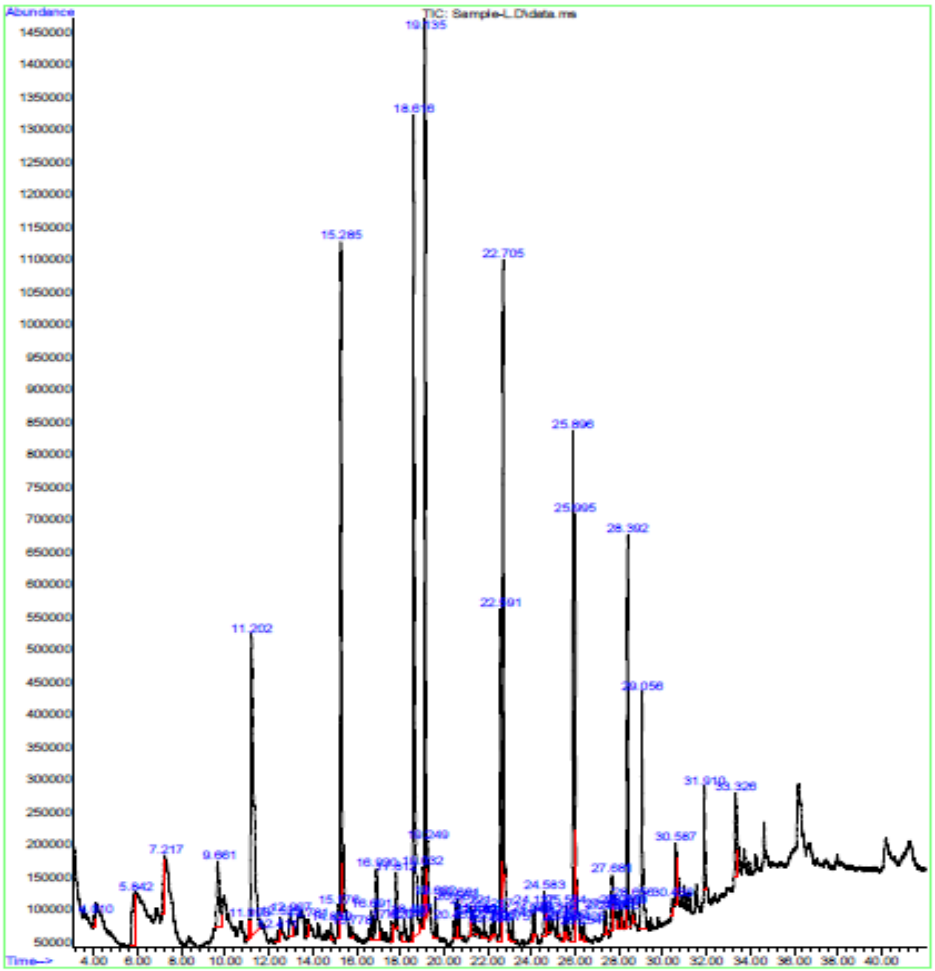
| Extracts | Concentration of extract/50µl mg/kg |
|---|---|
| Petroleum ether | 29.11 |
| Ethyl acetate | 41.71 |
| Ethanol | 23.304 |
| Methanol | 64.4425 |

### 3.2 Chromatogram of the Cinnamon powder extract by petroleum ether



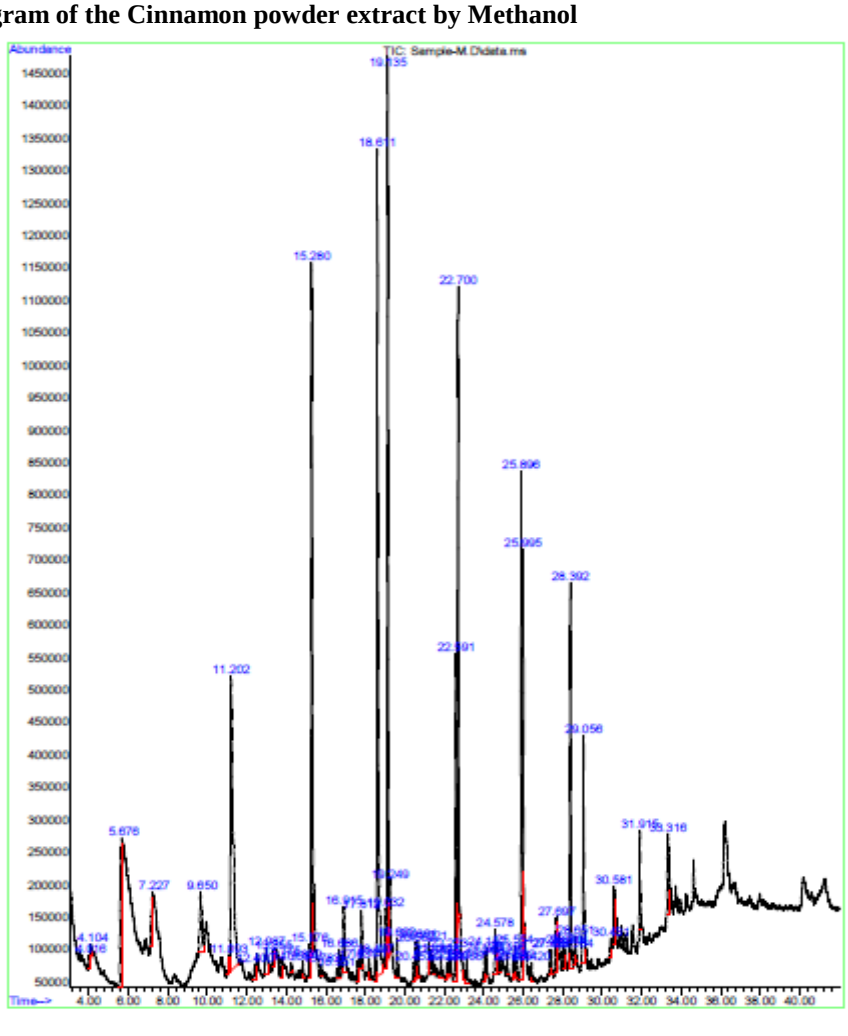
| Sl.no. | Compound name | RT | Area% |
|---|---|---|---|
| | Cinnamon powder extract by petroleum ether | | |
| 1 | Cyclododecane | 4.109 | 0.24 |
| 2 | 2-Propenal, 3-phenyl- | 5.520 | 5.64 |
| 3 | Cinnamaldehyde, (E)- | 5.520 | 5.64 |
| 4 | 2-Propenal, 3-phenyl- | 5.520 | 5.64 |
| 5 | alfa.-Copaene | 7.098 | 0.26 |
| 6 | Pentadecane, 1-methoxy-13-methyl- | 11.098 | 0.19 |
| 7 | Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl- | 11.098 | 0.19 |
| 8 | Cyclopentanecarboxylic acid, 3-methylbutyl ester | 11.098 | 0.19 |
| 9 | 17-Pentatriacontene | 14.258 | 0.17 |
| 10 | Cyclohexane, 1,2,4,5-tetraethyl- | 14.258 | 0.17 |
| 11 | 17-Pentatriacontene | 14.258 | 0.17 |
| 12 | Cyclohexane, 1,2,4,5-tetraethyl- | 14.258 | 0.17 |
| 13 | Heptadecane, 3-methyl- | 14.839 | 0.25 |
| 14 | Ethanol, 2-(tetradecyloxy)- | 14.839 | 0.25 |
| 15 | Lauric acid, 2-methylbutyl ester | 15.181 | 0.49 |
| 16 | Cyclohexanecarboxylic acid, 3-methylbutyl ester | 15.181 | 0.49 |
| 17 | Decanoic acid, 2-propenyl ester | 16.925 | 1.26 |
| 18 | Dodecane | 16.925 | 1.26 |
| 19 | Docosylpentafluoropropionate | 17.625 | 0.21 |
| 20 | Tetrapentacontane, 1,54-dibromo- | 18.186 | 0.46 |
| 21 | Cycloeicosane | 19.135 | 10.33 |
| 22 | 2-ethylbutyric Acid, 2,2,2-trifluoroethyl ester | 20.661 | 0.58 |
| 23 | Oxalic acid, isobutyl tetradecyl ester | 21.828 | 0.31 |
| 24 | Carbonic acid, octadecyl 2,2,2-trichloroethyl ester | 22.705 | 7.00 |
| 25 | Cycloundecane, (1-methylethyl)- | 24.578 | 0.63 |
| 26 | 17-Pentatriacontene | 26.425 | 0.23 |
| 27 | Fumaric acid, 3,5-difluorophenyl hexadecyl ester | 27.691 | 0.60 |

### 3.3 Chromatogram of the Cinnamon powder extract by Ethanol



| Sl.no. | Compound name | RT | Area% |
|---|---|---|---|
| | Cinnamon powder extract by Ethanol | | |
| 1 | Cinnamaldehyde, (E)- | 5.842 | 1.36 |
| 2 | 2-Propenal, 3-phenyl- | 5.842 | 1.36 |
| 3 | Pentadecane, 1-methoxy-13-methyl- | 11.093 | 0.30 |
| 4 | Cyclohexane, octyl- | 12.525 | 0.29 |
| 5 | Z-8-Hexadecene | 12.525 | 0.29 |
| 6 | Pentatriacontane, 13-docosenylidene | 14.839 | 0.24 |
| 7 | Oxalic acid, octadecyl propyl este | 14.839 | 0.24 |
| 8 | Octadecane, 1-(ethenyloxy)- | 14.839 | 0.24 |
| 9 | Decanoic acid, 2-propenyl ester | 16.920 | 1.61 |
| 10 | Dodecane | 16.920 | 1.61 |
| 11 | Tridecane | 16.920 | 1.61 |
| 12 | Eicosane, 9-cyclohexyl- | 17.620 | 0.18 |
| 13 | Tetracosylpentafluoropropionate | 17.620 | 0.18 |
| 14 | Heptacosyltrifluoroacetate | 17.620 | 0.18 |
| 15 | Ethanone, 1-(5,6,7,8-tetrahydro-2, 8,8-trimethyl-4H-cyclohepta[b]furan-5-yl)- | 17.812 | 0.61 |
| 16 | Cycloeicosane | 19.135 | 10.53 |
| 17 | Eicosylpentafluoropropionate | 19.602 | 0.63 |
| 18 | 2-Methyl-7-nonadecene | 19.602 | 0.63 |
| 19 | Cedranoxide, 8,14- | 19.602 | 0.63 |
| 20 | Cyclohexane, 1,1'-tetradecylidenebis | 20.453 | 0.19 |
| 21 | Cyclododecanemethanol | 20.453 | 0.19 |
| 22 | 1,1'-Bicyclohexyl, 4-methyl-4'-propyl | 20.453 | 0.19 |
| 23 | 1-Heneicosyl formate | 21.449 | 0.20 |
| 24 | 2- Chloropropionic acid, octadecyl ester | 21.449 | 0.20 |
| 25 | Triacontyl acetate | 21.449 | 0.20 |
| 26 | Butyl myristate | 25.896 | 6.23 |
| 27 | 1-Hexadecanol, 2-methyl- | 30.587 | 0.72 |

### 3.4 Chromatogram of the Cinnamon powder extract by Methanol



| Sl.no. | Compound name | RT | Area% |
|---|---|---|---|
| | Cinnamon powder extract by Methanol | | |
| 1 | 2-Dodecene, (E)- | 4.104 | 0.17 |
| 2 | 2-Propenal, 3-phenyl- | 5.676 | 3.69 |
| 3 | Cinnamaldehyde, (E)- | 5.676 | 3.69 |
| 4 | Phenol, 2,4-bis(1,1-dimethylethyl) | 9.650 | 1.60 |
| 5 | Carbonic acid, 2,2,2-trichloroethy l undec-10-enyl ester | 12.400 | 0.28 |
| 6 | Heptacosylpentafluoropropionate | 13.775 | 0.38 |
| 7 | Pentatriacontane, 13-docosenylidene | 14.839 | 0.22 |
| 8 | Trihexadecyl borate | 14.839 | 0.22 |
| 9 | 2-Pentadecanol | 15.176 | 0.43 |
| 10 | 8-Pentadecanone | 16.915 | 1.18 |
| 11 | Decanoic acid, 2-propenyl ester | 16.915 | 1.18 |
| 12 | 3-Acetylphenanthrene | 19.602 | 0.66 |
| 13 | Dimethylmalonic acid, 2,5-dichloro phenyl octadecyl ester | 21.818 | 0.30 |
| 14 | 17-Pentatriacontene | 22.134 | 0.17 |
| 15 | Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl- | 22.965 | 0.25 |
| 16 | Eicosyltrifluoroacetate | 22.965 | 0.25 |
| 17 | Carbonic acid, octadecyl 2,2,2-trichloroethyl ester | 23.151 | 0.32 |
| 18 | Cyclododecanemethanol | 24.049 | 0.35 |
| 19 | 9-Octadecenoic acid, (E)- | 25.196 | 0.22 |
| 20 | Cyclooctacosane | 33.316 | 2.12 |
| 21 | Octatriacontylpentafluoropropionate | 33.316 | 2.12 |
| 22 | Tetratriacontylpentafluoropropionate | 33.316 | 2.12 |

## IV. Conclusion

It may be suggested from the findingsof cinnamon extracts extracts can be used as a potential source of natural antimicrobial compound. Further research is needed for the identification of bioactive molecule present in cinnamon extracts and in vivo efficacy against food spoilage microorganisms before it is used for commercialization in the form of nutraceutical foods.

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