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GC-MS and FT-IR analysis of constituents of essential oil from *Cinnamon bark* growing in South-west of Ethiopia

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Abstract

The essential oil of cinnamon bark growing in Tepi (south-west, Ethiopia), was obtained by hydro distillation and gas chromatography-mass spectrometry was used to identify the component. Six components were identified and 2-propenal, 3-phenyl was the major constituent (87.013%) while Eugenol (9.317%), O-Methoxy cinnamic aldehyde (0.236%), α -muurolene (0.133%), Naphthalene, 1,2,3,4-tetrahydro-1,6-dime (0.195%), Tricyclo [3.3.1.0 (2,8)] nona-3,6-dien-9-on (0.173%), were minor constituents. 2-propenal, 3-phenyl comprises 87.013% of the essential oil extracted from cinnamon bark, and is the compound most responsible for the cinnamon barks' aroma and therapeutic effect.

Keywords: Essential oil, Cinnamon bark, Hydro distillation, Extraction.

1. Introduction

Essential oils (also called volatile or ethereal oils) ^[1] are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The essence or aromas of plants are due to volatile or essential oils, many of which have been valued since antiquity for their characteristic odors. The essential oils have characteristic fragrances and tastes are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters ^[2]. An estimated 3000 essential oils are known, of which about 300 commercially important are destined chiefly for the flavors and fragrances market ^[3]. It has long been recognized that some essential oils have antimicrobial properties ^[1, 4] and these have been reviewed in the past ^[5, 6] as have the antimicrobial properties of spices ^[5] but the relatively recent enhancement of interest in 'green' consumerism has lead to a renewal of scientific interest in these substances ^[6, 7]. Besides antibacterial properties ^[8-10], essential oils or their components have been shown to exhibit antiviral ^[11], antimycotic ^[12-15], antitoxigenic ^[16-18], antiparasitic ^[19, 20], and insecticidal ^[21, 22] properties. These characteristics are possibly related to the function of these compounds in plants ^[1, 23].

Due to their antimicrobial, insecticidal, antifungal, and antibacterial activities, essential oils have been intensely screened and applied in the fields of pharmacology, medical and clinical micro-biology, phytopathology and food preservation ^[24].

Techniques commonly employed for extracting essential oils include hydro distillation ^[25], steam distillation ^[26], solvent extraction ^[27], and supercritical fluid extraction. Hydro distillation or steam distillation is the most widely utilized physical method for isolating essential oils from the botanical material ^[28]. Although steam distillation is much popular for the isolation of essential oils on commercial scale and 93% of the oils are produced by this process, but it is not a preferred method in research laboratories ^[29]. This is probably due to unavailability of steam generators and suitable distillation vessels. Most studies which focus on the essential oil of herbs have made use of hydro distillation in Clevenger-type apparatus ^[30-32].

Several authors have compared the composition of essential oil obtained by hydro/steam distillation and the product obtained by super critical fluid extraction. They found that hydro/steam-distilled oil contained higher percentages of terpene hydrocarbons. In contrast, the super critical extracted oil contained a higher percentage of oxygenated compounds ^[33-34]. Khajeh *et al.*, (2004) ^[35] reported variation in the chemical composition of *Carum copticum* essential oil

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isolated by hydro distillation and supercritical fluid extraction methods. Silva *et al.*, (2004) ^[35] reported that essential oils from leaves of *Ocimum gratissimum*, *Ocimum micranthum* and *Ocimum selloi* obtained by steam distillation, microwave oven distillation and supercritical extraction with CO₂ showed different composition by GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS) is the most popular method for the determination of essential oil composition. Components existing in the essential oil can be identified by comparison of their relative retention time or indices and their mass spectra (MS). Identification of individual components of essential oils, however, is not always possible using MS data alone. Often different spectra are reported in a library for a single compound, with different common names, or systematic name, corresponding to an individual component sometimes apparent ^[36]. The spectral similarity of a great number of essential oil components causes difficulty in obtaining positive identification of individual components; mass spectra for sesquiterpenes are often identical or nearly identical ^[37]. Some authors have also evaluated different techniques for essential oil analysis, like the more comprehensive two-dimensional gas chromatography (GC×GC) ^[36, 38]. However, GC-MS analysis is still the most widely used method for routine

analysis of essential oils, and care must be taken to optimize the chromatographic conditions in order to obtain the most accurate results. The aim of the present research work is to extract the essential oil from cinnamon bark and subject to further analysis by GC-MS.

2. Methods and Materials

2.1 Plant material

The plant material (Cinnamon bark) was collected from local market, Tepi, in February 2013. Plant material was identified by Ethiopian Institute of Agricultural Research Tepi national Spice Research Center and transported to chemistry laboratory for essential oil extraction.

2.2 Hydro distillation (Extraction of essential oil)

300 gm of cleaned and dried plant material was powdered using metal mortar and pestle and placed in a round bottom flask fitted with condenser hydro distilled for 3hrs at atmospheric pressure and constant temperature. The strongly aromatic oil was separated from the water layer using diethyl ether and the solvent was removed by boiling (Fig.2.1).

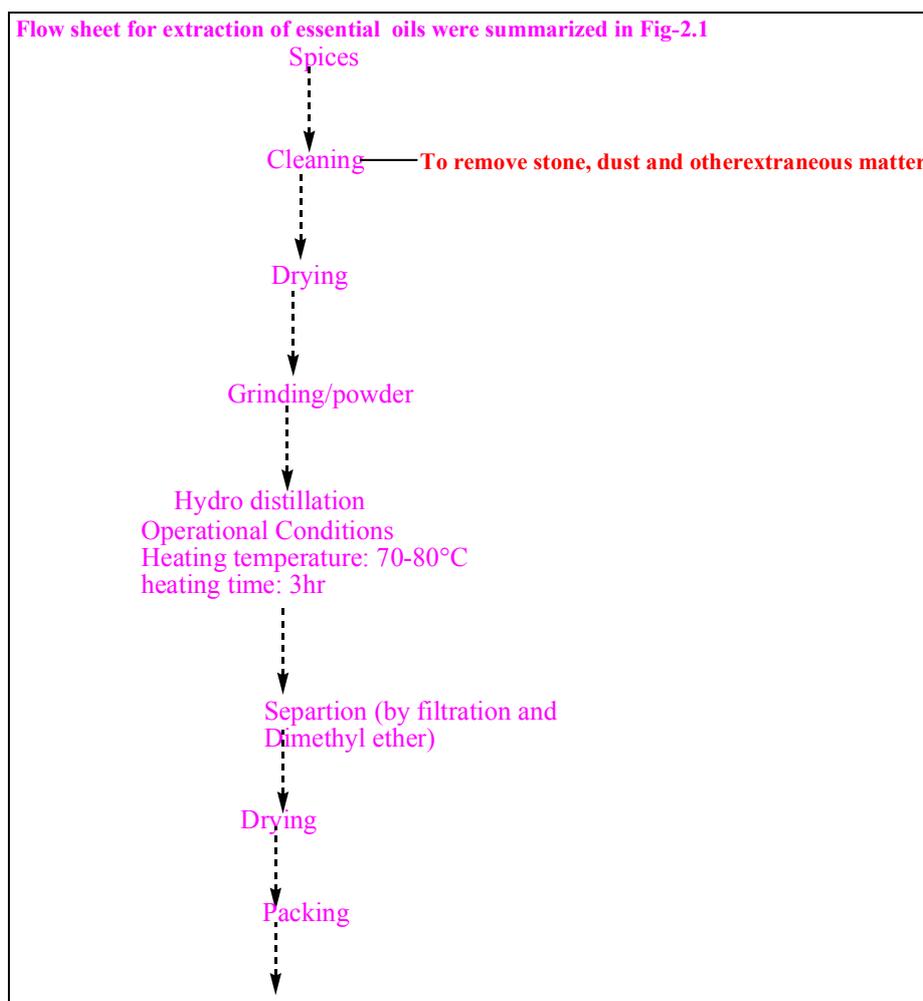


Fig 2.1: Process flow chart for extraction of essential oil

2.3 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrum of the essential oil was obtained using prinks

Elmer spectrum 65 FT-IR spectrometer in Addis Ababa University and functional groups were determined with the help of IR

correlation charts. The IR spectra were reported in % transmittance. The wave number region for the analysis was 4000-400 cm^{-1} (in the mid-infrared range).

3. GC-MS analysis

The component identification was achieved by the GC-MS analysis using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German) in Addis Ababa University. Helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 μl was employed, injector temperature 250 $^{\circ}\text{C}$; Ion-source temperature 280 $^{\circ}\text{C}$. The oven temperature was programmed from 50 $^{\circ}\text{C}$ (isothermal for 4min.), with an increase of 3 $^{\circ}\text{C}/\text{min}$, to 280 $^{\circ}\text{C}$ and held for 10min. isothermal at 280 $^{\circ}\text{C}$. Total GC running time was 90.67 min.

3.1 Identification of Components

The components of essential oil was identified on the basis of comparison of their retention time and mass spectra with published data [39, 40] and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system, in Addis Ababa University, Ethiopia. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

4. Results and Discussion

The retention times and chemical composition of phytochemicals present in cinnamon bark essential oil are presented in Table 1.

4.1 Determination of the functional groups present using FT-IR

The functional groups present in the essential oil were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer

with those of an IR correlation chart. In the FT-IR spectrum of *cinnamon bark* essential oil the absorption band or frequency from 3500 cm^{-1} – 3200 cm^{-1} broad (b), showed the presence of O-H stretch, H-bonded for alcohol and phenol and 3000 cm^{-1} -2850 cm^{-1} (medium (m) indicate C-H stretch for alkane. The absorbance band at 1666.90 cm^{-1} (m) revealed the presence of C=O bond for aldehyde. Due to the influence of conjugation and aromatic ring, the peak is wide than the normal cases of aldehyde compound. A strong absorption band between 900 cm^{-1} – 675 cm^{-1} indicated the presence of aromatic C=C. A medium-weak band between 1680-1600 cm^{-1} showed the presence of alkenes C=C stretch (Fig.3.1).

4.2 GC-MS chromatogram

A total of six different components, with different retention times, were eluted from the GC column as indicated by the chromatogram (Fig. 3.2) and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in fig. 3.3 to 3.8. The relative amount of individual components was calculated based on GC peak areas.

Comparison of the GC-MS spectrograph obtained with the instruments data bank together with computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system revealed that the essential oil of cinnamon bark contained different organic compounds that eluted at different retention times depending on the boiling point of the eluted component. The instruments data bank was also able to identify the presence of 2-propenal, 3-phenyl (87.013%), Tricyclo [3.3.1.0 (2,8)] nona-3,6-dien-9-on (0.173%), Eugenol (9.317%), O-Methoxy cinnamic aldehyde (0.236%), α -muurolene (0.133%), Naphthalene, 1,2,3,4-tetrahydro-1,6-dime (0.195%), with retention times of 30.037, 30.420, 32.903, 36.545, 38.218 and 39.208 minutes respectively.

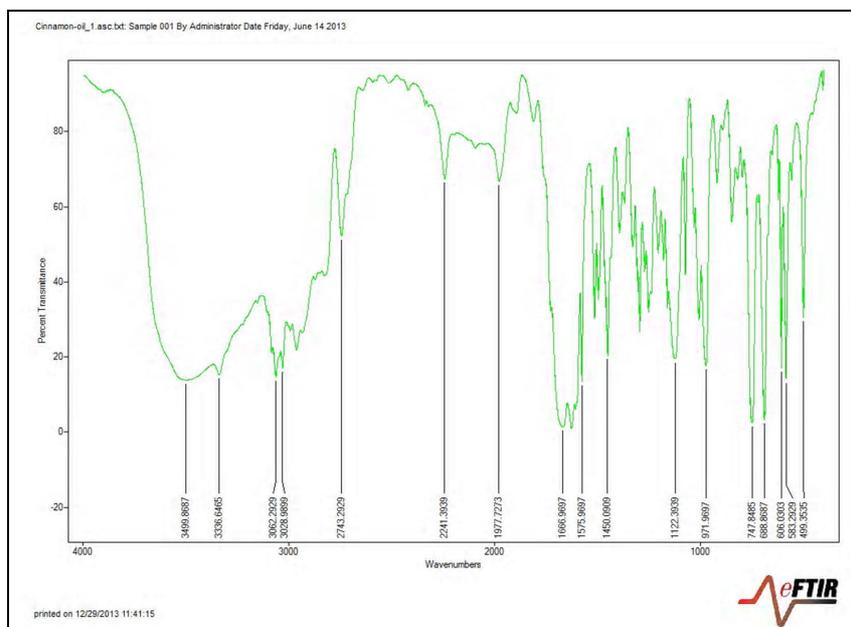


Fig 3.1: FT-IR spectra of cinnamon bark essential oil

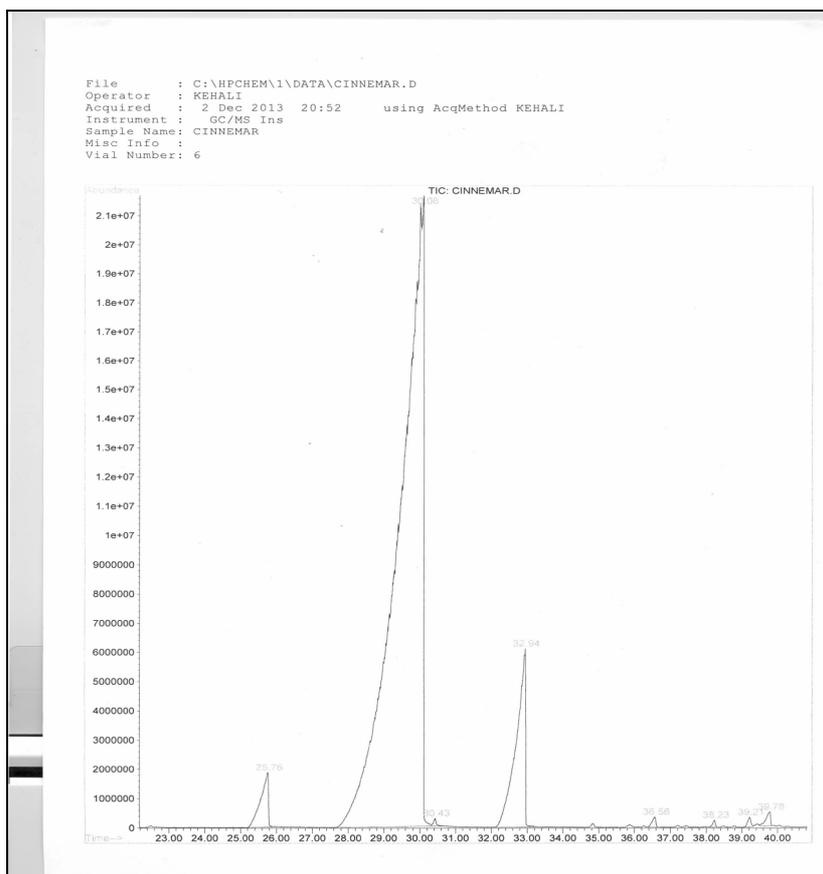


Fig 3.2: GC-MS profile of Cinnamon bark essential oil

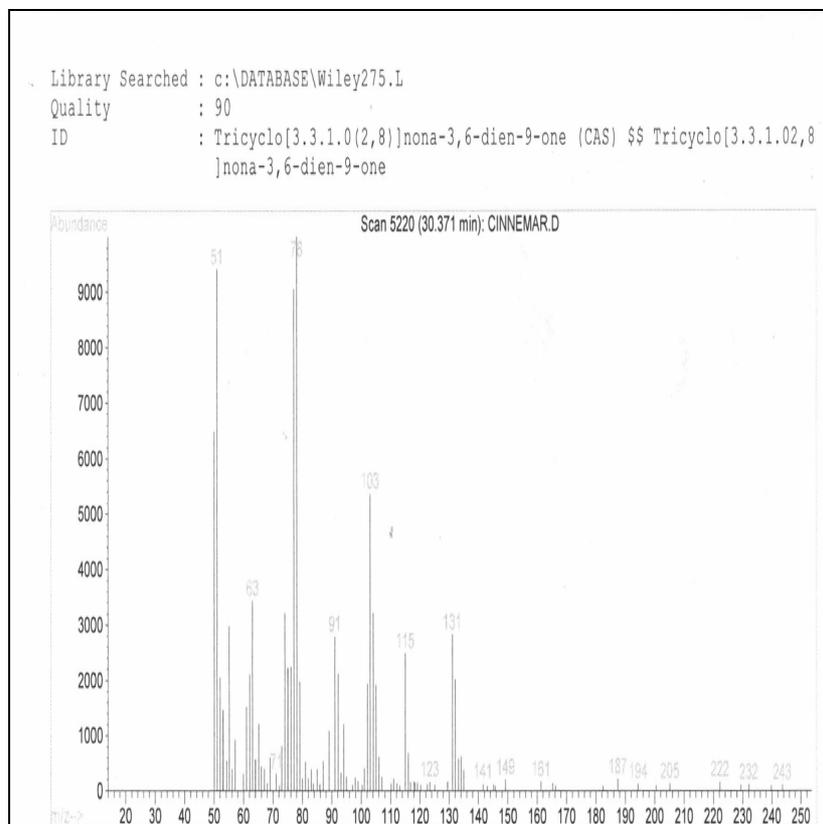


Fig 3.3: Mass profile of Peak at Rt 30.371 min; A GC- MS of peak eluted at Rt 30.371 min; Tricyclo [3.3.1.0 (2, 8)] nona-3, 6-dien-9-on,

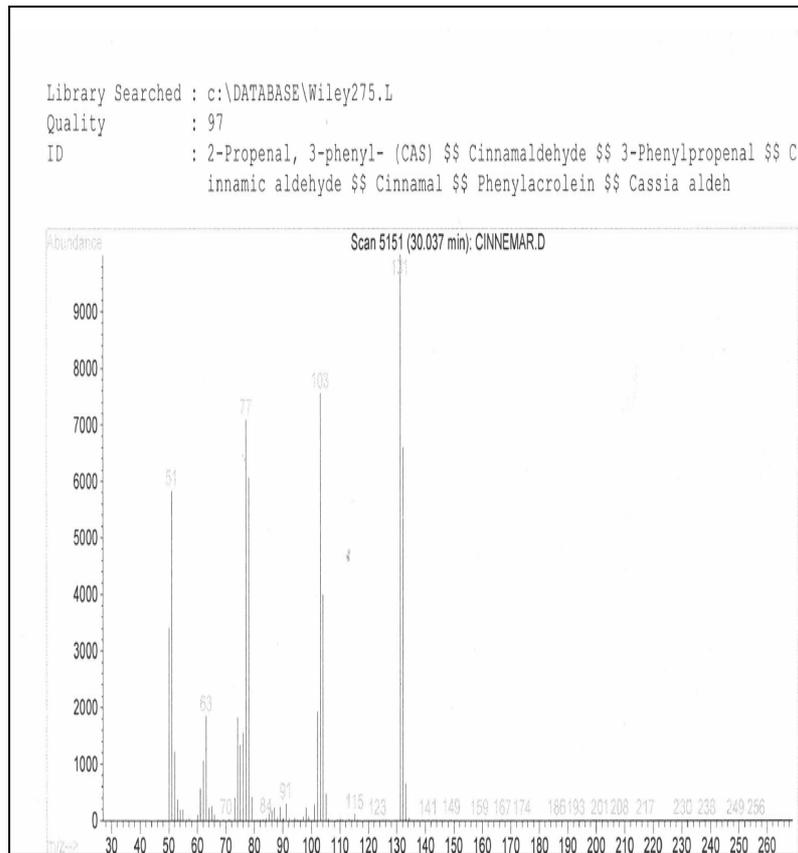


Fig 3.4: Mass profile of Peak at Rt 30.371min; A GC- MS of peak eluted at Rt 30.371 min; Tricyclo [3.3.1.0 (2, 8)] nona-3, 6-dien-9-on,

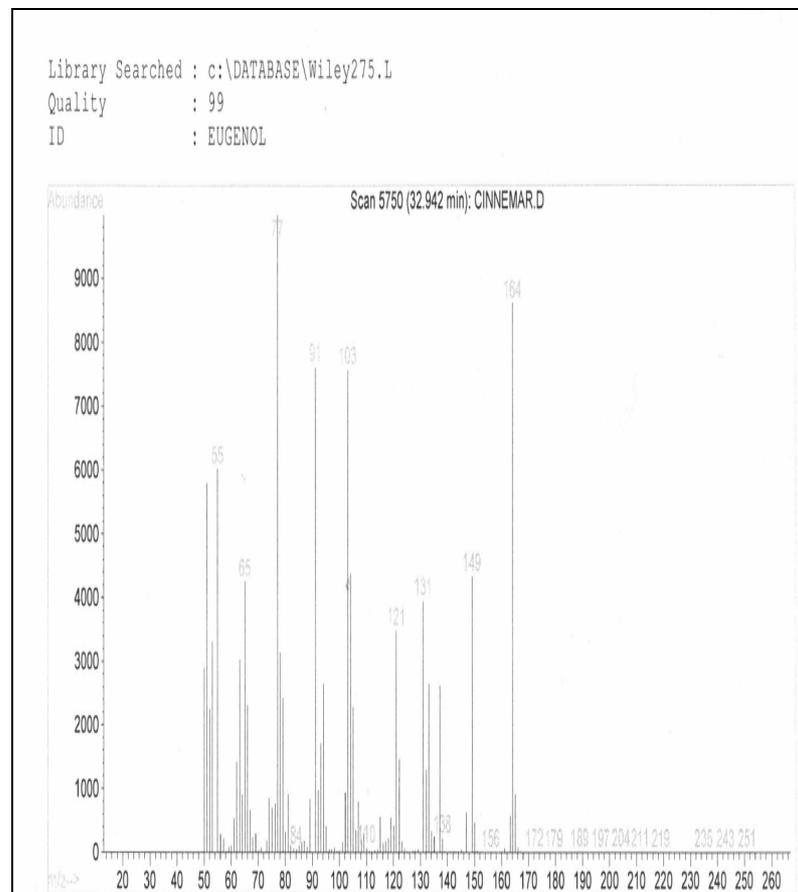


Fig 3.5: Mass profile of Peak at Rt 32.903min; A GC- MS of peak eluted at Rt 32.903 min; Eugenol

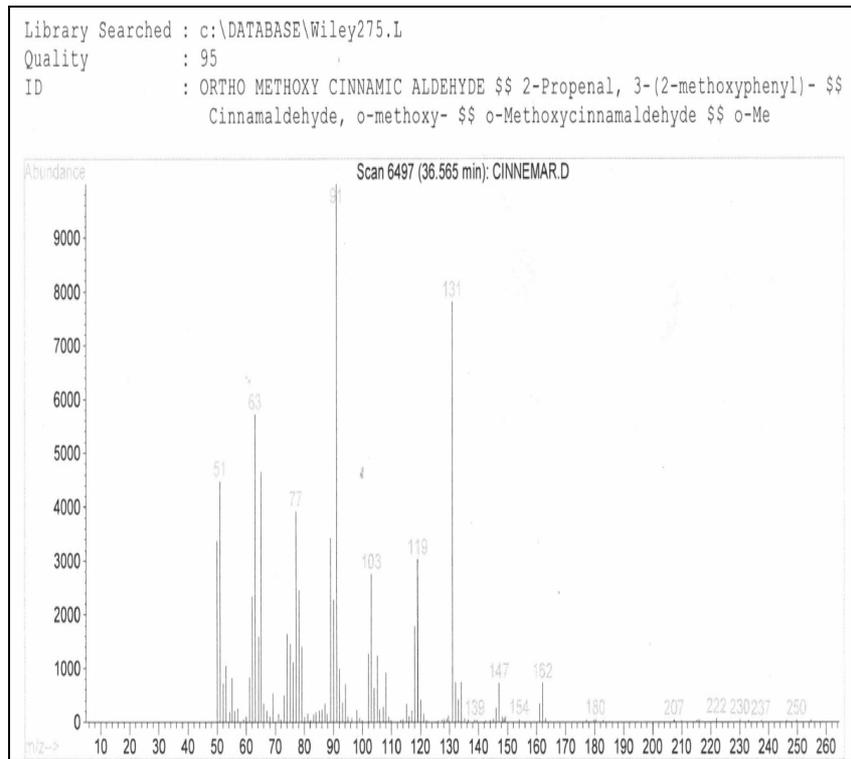


Fig 3.6: Mass profile of Peak at Rt 36.545 min; A GC- MS of peak eluted at Rt 36.545 min; O-Methoxy cinnamic aldehyde

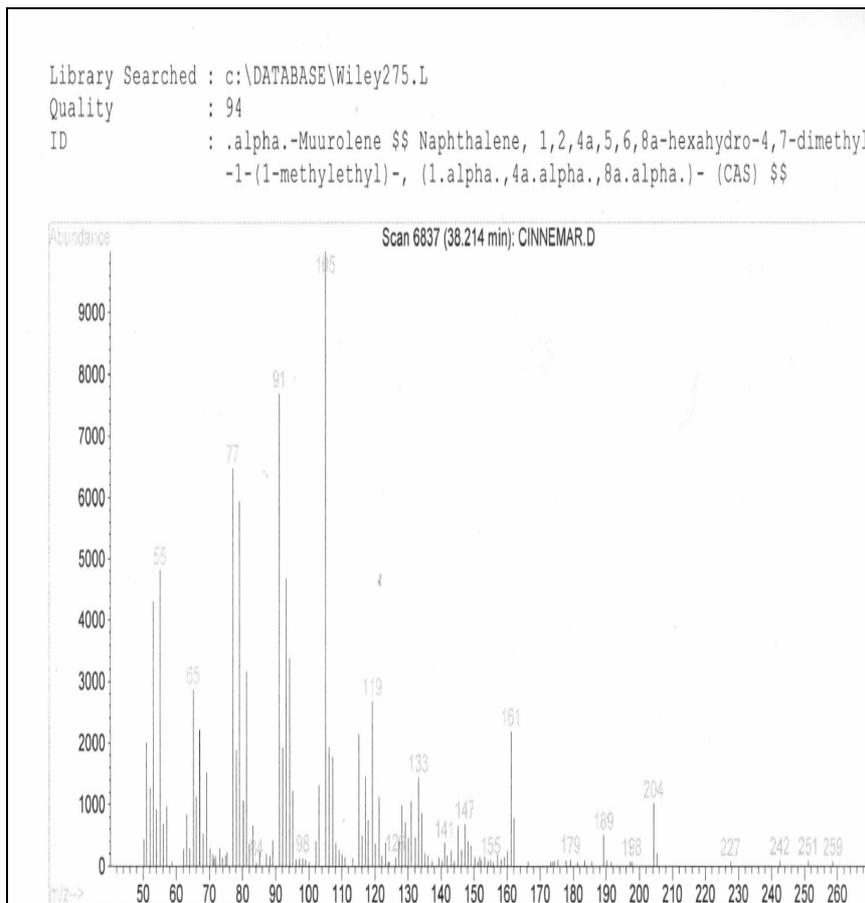


Fig 3.7: Mass profile of Peak at Rt 38.218 min; A GC- MS of peak eluted at Rt 38.218 min; α -muurolene

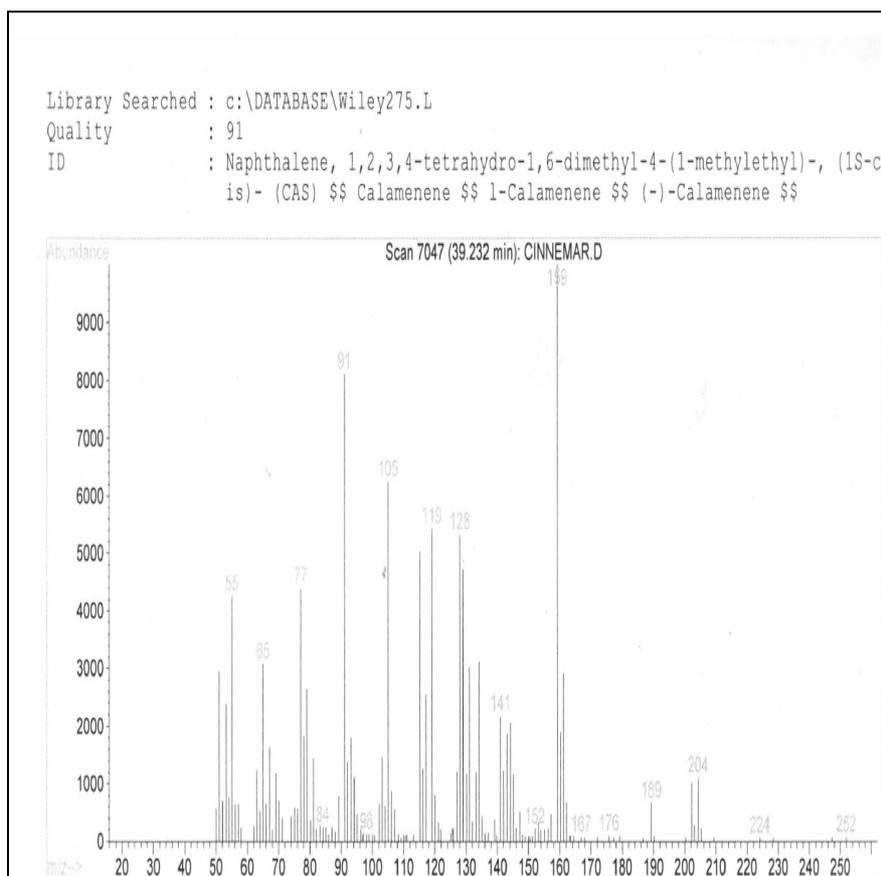
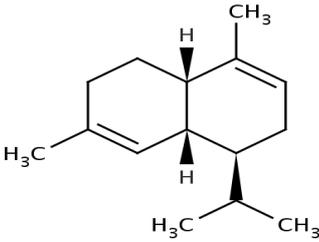
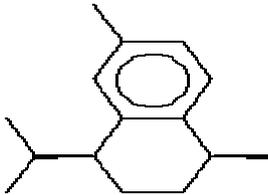


Fig 3.8: Mass profile of Peak at Rt 39.208 min; A GC- MS of peak eluted at Rt 39.208 min; Naphthalene, 1,2,3,4-tetrahydro-1,6-dime

Table 1: Phytocomponents identified in Cinnamon bark essential oils

| Rt (min.) | Compound | Structure | Molecular Formula | MW | Peak area |
|-----------|--|-----------|--|-----|-------------|
| 30.037 | 2-propenal, 3-phenyl | | C ₉ H ₈ O | 132 | 10129354187 |
| 30.420 | Tricyclo [3.3.1.0 (2,8)] nona-3,6-dien-9-on | | C ₉ H ₈ O | 132 | 20145271 |
| 32.903 | Eugenol | | C ₁₀ H ₁₂ O ₂ | 164 | 1084572327 |
| 36.545 | O-Methoxy cinnamic aldehyde | | C ₁₀ H ₁₀ O ₂ | 162 | 27423614 |

| | | | | | |
|--------|--|---|----------------|-----|----------|
| 38.218 | α -muurolene |  | $C_{15}H_{24}$ | 204 | 15480283 |
| 39.208 | Naphthalene, 1,2,3,4-tetrahydro-1,6-dime |  | $C_{15}H_{22}$ | 202 | 22686964 |

4.3 Phytochemical and their reported biological actions

The cinnamon spice is used for flavoring baked products. The bark and leaf oil are used in the manufacture of perfumes, soaps and toothpastes and also as a flavoring agent for liquors and in dentifrices. Besides these, cinnamon has a broad spectrum of medicinal and pharmacological application^[41]. Cinnamon possesses various biological activities such as antioxidant, antimicrobial, antidiabetic and antiallergic. For many centuries, cinnamon and its essential oil have been used as preservatives in food, due to the antioxidant property of cinnamon. *In vivo* lipid peroxidation causes tissue damage, which can lead to inflammatory diseases. Phenolic compounds, such as hydroxyl cinnamaldehyde and hydroxy cinnamic acid, present in the cinnamon extract, act as scavengers of peroxide radicals and prevent oxidative damages. Therefore the essential oil and possibly various extracts from cinnamon might be employed to retard autoxidation chain reactions in oils and fats^[42].

Cinnamon is reported to possess anti-inflammatory activity^[43]. The ethanolic extract (70%) of cinnamon was effective on acute inflammation in mice^[44]. An herbal ophthalmic preparation, called ophthacare containing 0.5% cinnamon was found to be effective as an anti-inflammatory in rabbits^[45]. Cinnamon extract has anti-diabetic effect. Cinnamon is reported to reduce the blood glucose level in non-insulin-dependent diabetics. Therapeutic studies have proved the potential of cinnamaldehyde and hydroxycinnamic acid as anti-diabetic agents. Cinnamaldehyde inhibits aldose reductase, a key enzyme involved in the polyol pathway. This enzyme catalyses the conversion of glucose to sorbitol in insulin-insensitive tissues in diabetic patients. This leads to accumulation of sorbitol in chronic complications of diabetes, such as cataract neuropathy and retinopathy. Aldose-reductase inhibitors prevent conversion of glucose to sorbitol, thereby preventing several diabetic complications^[46]. A decoction of dried twigs of cinnamon can produce an antipyretic effect in mice. Studies conducted in anaesthetized dogs and guinea pigs indicated that cinnamaldehyde or sodium cinnamate, also produced the hypothermic and antipyretic effects.

It also causes a hypotensive effect, which is due mainly to vasodilation of peripheral vessels. Cinnamaldehyde produced an analgesic effect in mice^[47]. Cinnamon oil showed an inhibitory effect against the Gram-positive bacteria *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus* and

Enterococcus faecalis, Gram-negative bacteria *Alcaligenes faecalis*, *Enterobacter cloacae*, *Escherichia coli*.

5. Conclusions

GC-MS analysis revealed that six different chemical components were identified in the essential oil of cinnamon bark, including 2-propenal, 3-phenyl (87.013%), Tricyclo [3.3.1.0 (2,8)] nona-3,6-dien-9-on (0.173%), Eugenol (9.317%), O-Methoxy cinnamic aldehyde (0.236%), α -muurolene (0.133%), Naphthalene, 1,2,3,4-tetrahydro-1,6-dime (0.195%). 2-propenal, 3-phenyl (87.013%) of the essential oil extracted from cinnamon bark, and is the compound most responsible for the cinnamon's aroma/fragrant odor. The quantity of phytochemical present in cinnamon bark that is grown Tepi (south west of Ethiopia) is different from other area. This is due to geographical position, cultivation, variety of cinnamon; harvesting time and extraction method affect the actual yield and composition of the essential oil^[48]. The ingredients obtained from this study indicate that the oil can be fully utilized for the manufacture of perfumery products, antimicrobial and antiseptic agents.

6. Acknowledgement

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