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Isolation & Characterization of Biologically Active Monoterpenes from Chloroform Extract of *C. camphora* through Bioactivity Guided Approach

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ABSTRACT

AIM- The aim of the present investigation is to isolate the active phytoconstituents through bioactive guided approach from the chloroform extract. **MATERIAL & METHODS-** The fresh leaves of *C. camphora* were collected from outfield during the month of July that shows the green color with rough surface. Defatted drug was subjected to extraction with chloroform, ethyl acetate, ethanol and finally by using water. Preliminary phytochemical screening of different extracts for the presence of various active phytoconstituents was performed. Chloroform extract (50 g) was dissolved in a minimum volume of chloroform and adsorbed on silica gel (60-120 mesh), dried and applied on the column to separate possible phytoconstituents. Column was first eluted with pure n-hexane and then gradually with increasing quantity (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35 and finally 50:50) of ethyl acetate. The fractions showing same TLC pattern were pooled together and finally 5 fractions (F1-F5) were obtained. On In vitro anti-diabetic evaluation of all collected fractions, Fraction-2 (F2) showed highest activity and single spot in TLC, it showed the strong presence of terpenes. **RESULTS & DISCUSSION-** One of the distinctive features in the IR spectrum of Linalool is the presence of a sharp and intense peak in the range of 3400 cm^-1, indicative of the O-H stretching vibrations associated with the hydroxyl group. This peak signifies the alcohol functionality inherent in Linalool. One prominent feature in the IR spectrum of borneol is the broad peak in the region around 3400 cm^-1, indicative of O-H stretching vibrations. The singlet at δ 1.39 corresponds to three equivalent protons in a methyl group, indicating the presence of aliphatic methyl moieties in linalool. **CONCLUSION-** From the chloroform extract, two active phytocompounds i.e. Borneol and Linalool were isolated and characterized.

KEYWORDS- Borneol, Linalool, *C. camphora*, Bioactivity Guided Isolation, Chloroform extract.

INTRODUCTION

About two to three decades ago, most of the drugs were obtained from natural sources. Herbal plants have been used for the treatment of various disorders with no sound scientific knowledge on its function, phyto-chemistry, and adverse effects [1]. Medicinal plants play an important role in both preventive and curative medicinal preparations for human beings. Herbal medicines are the only affordable source of healthcare, especially for the poorest patients [2]. Furthermore, herbal medicines are gaining popularity both in developing and developed countries due to their safety, efficacy, quality, very low adverse effects, and easy availability. Some of the currently available drugs such as aspirin, digitalis, quinine (anti-malarial), vincristine, and vinblastine (anti-cancerous) were derived from the plant sources. Plant-derived phytochemicals have beneficial effect against diabetes, microorganism, inflammation, cardiovascular diseases, blood disorders, cerebral disorders, immune system, oxidative stress, reproductive disorder, and cancer chemotherapy [3]. According to the World Health Organization (WHO), more than 21,000 plants are used for medicinal purposes in the world [4].

C. camphora (L.) is a renowned Unani medicinal herb applied for several disease conditions in Unani as well as other traditional medicines. Since the ancient era in the Unani traditional system of medicine, *C. camphora* has been using its ethnomedicinal properties like antiseptic, analgesic, and rubefacient properties. Camphor has been use for very long time in various traditional systems of medicine such as Ayurveda, Unani, Siddha, and Chinese. It has been used in Unani medicine mainly in respiratory disorders, gastrointestinal, integument disease, eye diseases, and nervine and cerebral disorders especially in hot conditions for headache, strengthening senses and brain [5], bilious diarrhea [6], inflammation of the liver [5], and useful in bladder and kidney inflammation [7]. Furthermore, externally, it is used for various ailments such as eye diseases, ear pain, joint, muscular pain, chest congestion, and headache applications such as ear drops or gargling with or without other suitable drugs [8-10]. In our previous study, the chloroform extract of *C. camphora* leaf were evaluated for the In vitro anti-diabetic activity. Since there is no scientific evidence for the isolation of active phytoconstituents from the above said extract so our focus of the study is to isolate the active phytoconstituents through bioactive guided approach.

MATERIAL & METHODS

Collection of Leaves of *C. camphora*

The fresh leaves of *C. camphora* were collected from outfield during the month of July that shows the green color with rough surface. The plant leaves and barks were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plant was identified by the Botanist and herbarium specimen was submitted in Department of Pharmacognosy.

Successive Extraction Methods

Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mare was dried in air to remove traces of petroleum ether. Defatted drug was subjected to extraction with chloroform, ethyl acetate,

ethanol and finally by using water. The % Yield of the Petroleum ether, chloroform, ethyl acetate, ethanol, & aqueous extract was calculated by using the respective formula [11].

Phytochemical Screening

Preliminary phytochemical screening of different extracts for the presence of various active phytoconstituents was performed [12].

Bioactive Guided Isolation of Active Phytoconstituents

The slurry of adsorbent (silica gel; 60-120 mesh) was prepared by mixing the adsorbent in the n-hexane and used as stationary phase. It was then poured into glass column (90cm x 3cm) and allowed to settle. Chloroform extract (50 g) was dissolved in a minimum volume of chloroform and adsorbed on silica gel (60-120 mesh), dried and applied on the column to separate possible phytoconstituents. The combinations of solvent systems developed for TLC was used as mobile phase for column chromatography and column was eluted by gradient elusion methods. Column was first eluted with pure n-hexane and then gradually with increasing quantity (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35 and finally 50:50) of ethyl acetate. Total 185 fractions were collected of 25 ml elutes. The column was eluted till 90 % of fraction loaded eluted out. All the collected fractions were monitored simultaneously on a TLC plate using n-hexane: ethyl acetate (79:21) as solvent system. The fractions showing same TLC pattern were pooled together and finally 5 fractions (F1-F5) were obtained. Percentage yield of collected elutes were determined in respect to the total weight of the fraction. On In vitro anti-diabetic evaluation of all collected fractions, Fraction-2 (F2) showed highest activity and single spot in TLC, it showed the strong presence of terpenes. As per the previous literature survey, terpenes are the most active phytoconstituents which had most promising antidiabetic, anti-oxidant and hypolipidemic activity [13].

CHARACTERIZATION AND IDENTIFICATION OF COMPOUNDS

The structure was characterized by means of IR, NMR, and MASS spectral analysis for the structure determination and their identity [14].

RESULTS & DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of different extracts showed the presence of steroids in petroleum ether, terpenes and terpenoids in chloroform, some minor flavonoids and phenolic compounds in ethyl acetate and ethanol extracts.

Characterization of Linalool

IR Spectra of Linalool

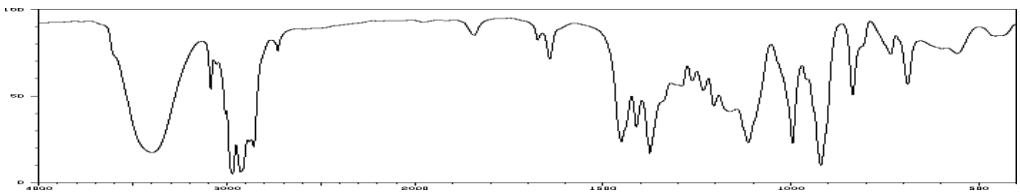

Figure No. 1: IR Spectra of Linalool

Table No. 1: Characterization of Linalool by IR peaks

Functional Group	**Frequency Range (cm^-1)**	**IR Peak**
O-H (Hydroxyl)	3400	Sharp Peak
C-H (Aliphatic)	2850-3000	Sharp Peaks
C=C (Alkene)	~1650	Medium Peak
C-O (Ether)	1250-1000	Medium Peaks
C-H Bending	1300-900	Various Peaks

Linalool, a monoterpene alcohol, is renowned for its pleasant aroma and is commonly found in various essential oils. One of the distinctive features in the IR spectrum of Linalool is the presence of a sharp and intense peak in the range of 3400 cm^-1, indicative of the O-H stretching vibrations associated with the hydroxyl group. This peak signifies the alcohol functionality inherent in Linalool. The C-H stretching vibrations of the aliphatic hydrocarbon chains are typically observed in the region of 2850-3000 cm^-1. The fingerprint region of the spectrum, ranging from 1500 to 500 cm^-1, provides specific information about Linalool's functional groups. Notably, the C=C stretching vibrations of the double bond in the terpene structure may manifest around 1650 cm^-1. Additionally, the C-O stretching vibration is usually evident in the range of 1250-1000 cm^-1, emphasizing the presence of oxygen in the compound. Further, the IR spectrum of Linalool may display characteristic peaks related to the bending vibrations of C-H bonds, appearing in the 1300-900 cm^-1 range. These vibration modes contribute to the overall fingerprint of Linalool, aiding in its identification and structural elucidation.

H^1NMR spectrum of Linalool

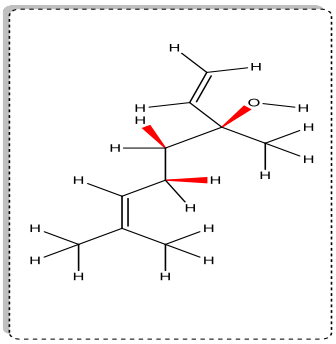

Figure No. 2: Structure of Linalool

^{1}H NMR: δ 1.39 (3H, s), 1.49-1.66 (8H, 1.54 (s), 1.54 (s), 1.60 (t, *J* = 7.4 Hz), 1.60 (t, *J* = 7.4 Hz)), 1.96-2.10 (2H, 2.03 (td, *J* = 7.4, 7.1 Hz), 2.03 (td, *J* = 7.4, 7.1 Hz)), 4.94-5.15 (2H, 5.02 (dd, *J* = 16.4, 1.4 Hz), 5.08 (dd, *J* = 10.9, 1.4 Hz)), 5.23 (1H, t, *J* = 7.1 Hz), 5.88 (1H, dd, *J* = 16.4, 10.9 Hz). The singlet at δ 1.39 corresponds to three equivalent protons in a methyl group, indicating the presence of aliphatic methyl moieties in linalool. The range from δ 1.49 to 1.66 encompasses eight protons in various aliphatic methylene groups. Within this range, there are signals at 1.54 (s), 1.54 (s), 1.60 (t, J = 7.4 Hz), and 1.60 (t, J = 7.4 Hz), revealing the complexity of the aliphatic chain with both singlet and triplet features. The region from δ 1.96 to 2.10 involves two protons in aliphatic methine groups, exhibiting a doublet of doublet pattern at 2.03 (td, J = 7.4, 7.1 Hz) and

2.03 (td, J = 7.4, 7.1 Hz). This suggests a coupling interaction with neighboring protons. The signals at δ 4.94-5.15 represent two protons in an aromatic environment, with a doublet of doublet pattern at 5.02 (dd, J = 16.4, 1.4 Hz) and 5.08 (dd, J = 10.9, 1.4 Hz). This indicates coupling with adjacent protons. The signal at δ 5.23 corresponds to a proton in an olefinic (double bond) environment, displaying a triplet pattern with a coupling constant of J = 7.1 Hz. The peak at δ 5.88 represents a proton in an olefinic environment, displaying a doublet of doublet pattern with coupling constants J = 16.4 and 10.9 Hz.

13C NMR spectrum of Linalool

The presence of a singlet at 17.9 ppm suggests the existence of a carbon environment where the carbon atoms are not strongly influenced by nearby atoms or functional groups. This may correspond to a methyl or methylene group. A singlet at 22.8 ppm indicates another isolated carbon environment, possibly associated with a different type of methyl or methylene group in the linalool molecule. The singlet at 25.8 ppm implies the presence of a unique carbon environment with minimal neighboring influences, potentially associated with a specific functional group or carbon-carbon arrangement. This singlet at 27.9 ppm suggests another distinct carbon environment, potentially representing a specific structural feature within the linalool molecule. The singlet at 40.9 ppm indicates a carbon environment that is relatively shielded or less influenced by neighboring atoms. This could be associated with a different type of carbon arrangement or functional group. The singlet at 73.5 ppm suggests the presence of a carbon environment that may be associated with a distinct functional group or a unique carbon-carbon arrangement in linalool. This singlet at 113.2 ppm represents a carbon environment that is distinct from others in the molecule, potentially associated with a specific functional group or structural motif. The singlet at 124.2 ppm suggests a unique carbon environment, possibly associated with a functional group or a specific carbon-carbon arrangement within the linalool molecule. This singlet at 132.0 ppm indicates the presence of a distinct carbon environment, potentially associated with a specific functional group or structural feature in linalool. The singlet at 145.4 ppm represents a carbon environment that is likely associated with a unique functional group or a specific carbon-carbon arrangement within the linalool molecule.

Mass Spectrum of Linalool

Mass spectrum of Linalool Interpreting mass spectra involves analyzing the peaks and identifying the fragments produced during the fragmentation of a molecule. Linalool is a compound commonly found in essential oils, such as lavender, and is used in various products for its pleasant aroma. Linalool is a terpene alcohol with the molecular formula C10H18O. The base peak ar m/z is 154 represent the molecular ion (M+) of linalool. The identified fragment and characteristic peaks suggest the presence of common functional in linalool such as methyl, ethyl , hydroxyl, and alkyl group.

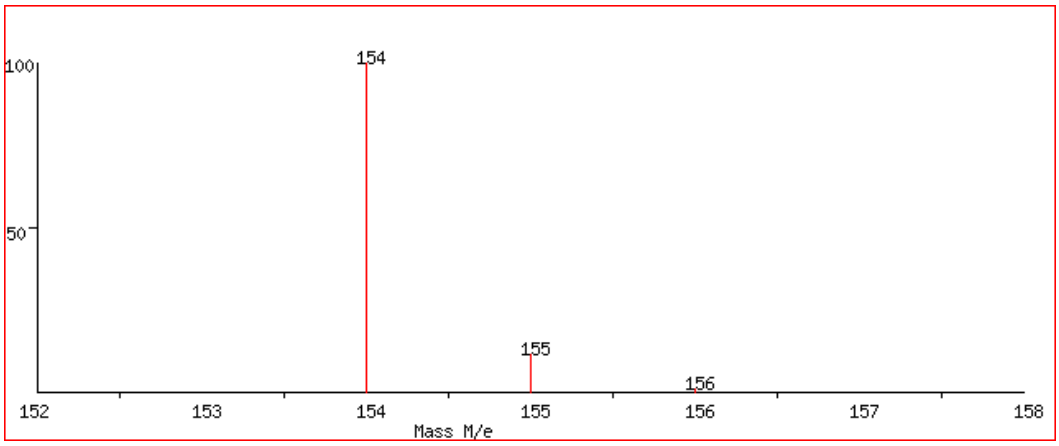

Figure No. 3: Mass Spectra of Linalool

The Molecular Ion Peak base peak at m/z 154 represents the molecular ion (M+) of linalool. The identified fragments and characteristic peaks suggest the presence of common functional groups in linalool, such as methyl, ethyl, hydroxyl, and alkyl groups. It's important to note that the interpretation of mass spectra is complex and often requires additional information, such as isotopic patterns, to confidently determine the structure of a compound. The interpretation provided here is a simplified example and may not capture all nuances of a real mass spectrum.

Characterization of Borneol

IR spectra of Borneol

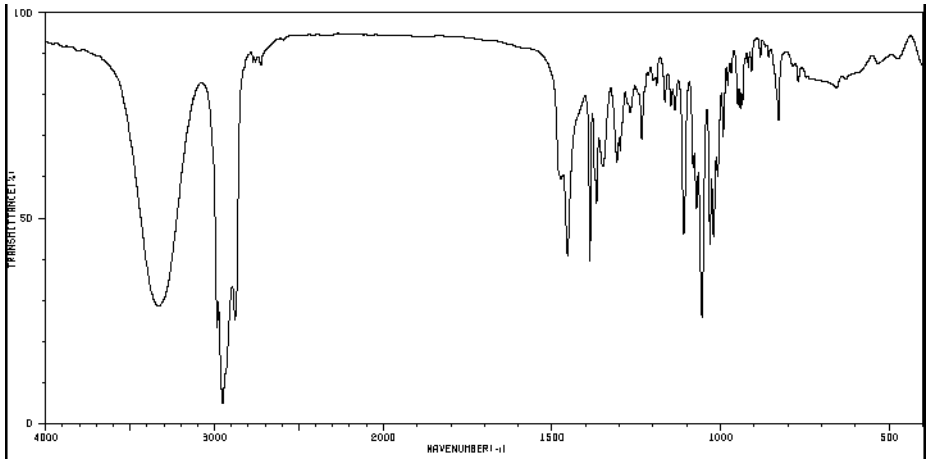

Figure No. 4: IR Spectra of Borneol

Table No. 2: Characterization of Borneol by IR peaks

Functional Group	**Frequency Range (cm^-1)**	**IR Peak**
O-H (Hydroxyl)	3400	Broad Peak
C-H (Aliphatic)	2850-3000	Sharp Peaks
C-O (Ether)	1250-1000	Medium to Sharp Peaks
C=C (Alkene)	~1650	Medium Peak
C-H Bending	1300-900	Various Peaks

Borneol is a bicyclic organic compound that belongs to the class of terpenoids. Its infrared (IR) spectrum provides valuable insights into its molecular structure and functional groups. One prominent feature in the IR spectrum of borneol is the broad peak in the region around 3400 cm^- 1, indicative of O-H stretching vibrations. This corresponds to the hydroxyl group present in borneol, a characteristic feature of many terpenoid compounds. Additionally, the C-H stretching vibrations of the aliphatic hydrocarbon chains are observed in the region of 2850-3000 cm^-1. The fingerprint region of the spectrum (1500-500 cm^-1) provides specific information about the functional groups present in borneol. Notably, the presence of the C-O stretching vibration appears in the vicinity of 1250-1000 cm^-1, confirming the existence of oxygen-containing moieties in the molecule. Borneol may also exhibit peaks associated with the bending vibrations of C-H bonds in the 1300-900 cm^-1 range.

1H NMR spectrum of Borneol

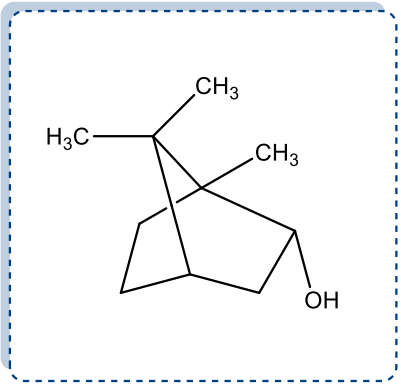

Figure No. 5: Structure of Borneol

^{1}H NMR: δ 0.85-1.02 (9H, 0.90 (s), 0.90 (s), 0.97 (s)), 1.20-1.94 (6H, 1.29 (dddd, *J* = 13.0, 8.1, 4.9, 4.2 Hz), 1.42 (ddd, *J* = 16.0, 8.1, 4.2 Hz), 1.52 (ddd, *J* = 16.0, 8.1, 4.2 Hz), 1.65 (dddd, *J* = 13.0, 8.1, 4.2, 1.4 Hz), 1.79 (ddd, *J* = 13.9, 8.1, 4.9 Hz), 1.87 (ddd, *J* = 13.9, 4.2, 1.4 Hz)), 2.14 (1H, tt, *J* = 4.9, 1.4 Hz), 3.49 (1H, dd, *J* = 8.1, 4.2 Hz). This region encompasses three singlets at 0.90 ppm, 0.90 ppm, and 0.97 ppm, representing a total of nine equivalent protons in aliphatic methyl groups. The singlet nature suggests that these methyl groups are not influenced significantly by neighboring protons. Multiple peaks in this region denote the presence of aliphatic methylene and methine groups. A complex multiplet at 1.29 ppm (dddd, J = 13.0, 8.1, 4.9, 4.2 Hz) corresponds to two protons. A triplet at 1.42 ppm (ddd, J = 16.0, 8.1, 4.2 Hz) represents a specific methine group. Another triplet at 1.52 ppm (ddd, J = 16.0, 8.1, 4.2 Hz) indicates a different methine group. A complex multiplet at 1.65 ppm (dddd, J = 13.0, 8.1, 4.2, 1.4 Hz) corresponds to two protons. A triplet at 1.79 ppm (ddd, J = 13.9, 8.1, 4.9 Hz) represents a specific methine group. Another triplet at 1.87 ppm (ddd, J = 13.9, 4.2, 1.4 Hz) indicates a different methine group. The singlet at 2.14 ppm corresponds to a specific methylene group, indicating a unique chemical environment for this proton. The doublet of doublet at 3.49 ppm (dd, J = 8.1, 4.2 Hz) suggests the presence of an olefinic (double bond) proton, indicating a specific unsaturation in the borneol structure.

13C NMR SPECTRUM

The singlet at 25.0 ppm suggests the presence of a unique carbon environment, possibly associated with a specific functional group or a distinct carbon-carbon arrangement. These singlets at 25.6-25.7 ppm indicate the existence of two carbon environments, each with minimal neighboring influences. This could be indicative of distinct methyl groups or other aliphatic carbons. The singlet at 26.6 ppm represents another isolated carbon environment, possibly associated with a specific functional group or unique carbon-carbon arrangement. These singlets in the range of 30.0 to 40.6 ppm indicate the presence of distinct carbon environments, likely associated with various aliphatic carbons or specific functional groups within the borneol molecule. The singlet at 74.0 ppm represents a carbon environment that is unique and could be associated with a specific functional group or structural feature in borneol.

Mass spectrum of Borneol

The mass spectrum of the unknown compound exhibited a peak at 154 *m/z* , confirming that the molecule was an oxygenate, but the retention time of the unknown compound was shorter than the other alcohol products.

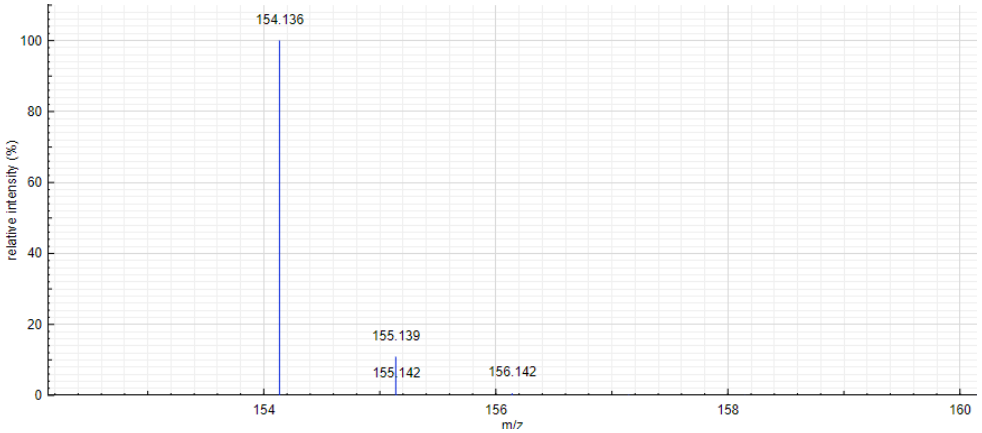

Figure No. 6: *Mass spectrum of Borneol*

Borneol is a bicyclic alcohol with the molecular formula $C_{10}H_{18}O$. The base peak at m/z 154 represents the molecular ion (M+) of borneol. The identified fragments and characteristic peaks suggest the presence of common functional groups in borneol, such as methyl, ethyl, hydroxyl, and cyclic groups.

CONCLUSION

Previous study reported that *C. camphora* has therapeutic applications in various diseases and its proven in few recent preclinical and clinical studies. From the above study, the two monoterpenes were isolated and characterized by various spectral techniques. Furthermore, dose range, route of administration, and dose frequency must be clarified in the upcoming research. However, more preclinical trials are recommended to explore its therapeutic applications in other diseases. Furthermore, it has been normally combined with other single drugs in Unani medicine; hence, drug interactions should be researched further in conventional therapies.

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