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Essential Oil Extraction and Characterization of Seeds of Chrozophora Tinctoria

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Abstract

Essential oil is complex mixture of volatile organic compounds that are derived from terpenoids and alkaloids through biosynthetic pathways, including the phenylpropene, methylerythritol, and mevalonate pathways. This oil is valuable in pharmaceuticals and can act as herbicides, pesticides, antimicrobials, or anticancer agents. The study aimed to extract oil from the seeds of Chrozophora tinctoria using the Clevenger apparatus and to characterize the extracted oil through UV and FTIR spectroscopy. This analysis focused on determining the chemical composition and physical properties of the oil, identifying functional groups, and detecting potential bioactive compounds. In Sample 1, the study identified eight peaks corresponding to specific functional groups, such as aldehydes, ketones, amines, esters, ethers, and aromatic structures. These functional groups were determined based on characteristic vibrations, including C-H bending, C-N stretching, and C-O stretching, which provides insight into the sample's chemical composition. On the other hand, sample 2, exhibited 14 peaks, suggesting a more complex mixture with functional groups like bromoalkenes, ethers, esters, and aromatic rings. The spectral analysis of this sample revealed vibrations such as C-Cl and C-Br stretching, along with C-O and O-H stretching, indicating the presence of various organic compounds. This characterization provides valuable insights into the oil's chemical properties, supporting its potential for further research, industrial applications, and the development of valuable products from Chrozophora tinctoria.

Keywords: Essential oil, Clevenger apparatus, UV, and FTIR

Introduction

The aroma and fragrance industry represents an $18 billion annual market, with the international trade of essential oils experiencing an average growth of 10% per year. However, the use of heavy metals in catalytic processes has become a significant concern (Schwab, Davidovich-Rikanati et al. 2008). According to

Professor Dr. Gerhard Buchbauer of the Institute of Pharmaceutical Chemistry, University of Vienna:
"Essential oils are more or less volatile substances with more or less odorous impact, produced either by steam distillation, dry distillation, or mechanical treatment from a single species." (CLEYETMERLE, ZIEGLER et al. 1994).
Essential oils are crucial raw materials in the fragrance industry. These oils are intricate blends of organic compounds produced by living organisms and extracted exclusively through physical methods such as pressing and distillation. Essential oils are derived from plant sources and can be described as concentrated, hydrophobic liquids containing highly volatile organic aromatic compounds. They are extracted from various parts of plants, including flowers, fruits, leaves, and bark, and are stored in specialized structures such as oil cells, glandular trichomes, and resin ducts. These oils contain defensive compounds that protect against herbivours and act as internal chemical messengers within plants. Not only do they function as natural herbicides they also attract pollinators, facilitating pollination. Like other secondary metabolites, these compounds play a crucial role in a plant's self-defense and survival under harsh environmental conditions. All plants have the ability to produce volatile aromatic compounds.
The use of essential oils as disinfectants, antioxidants, and food preservatives is of great importance from a food science perspective (Burt 2004) (Rohman, Che Man et al. 2010) (EL KOLLI 2018). In recent years, the use of natural antioxidants derived from plants has gained significant attention in the food industry and preventive medicine, as they are considered safer and possess health benefits. The potential of essential oils in treating infectious diseases is also being investigated. Advanced research is underway to explore their medical applications and effectiveness against
various infectious diseases as alternative medicines (Kiarostami, Bahrami et al. 2009) (Rohman, Che Man et al. 2010) (Politeo, Jukic et al. 2007).
The extensive use of essential oils in the pharmaceutical, cosmetics, food, and beverage industries highlights the need to explore and characterize new plant sources of essential oils (Delaquis, Stanich et al. 2002). Herbs are widely recognized as valuable sources of volatile components and aromatic compounds. Various studies have demonstrated that herbs containing antioxidants and other natural pharmaceutical components contribute positively to health by reducing degenerative processes and addressing the underlying causes of certain diseases due to their beneficial properties(Shahidi 1997) (Gilani 2005) (Sahib, Anwar et al. 2013). Natural extracts from therapeutic herbs contain numerous organic compounds that play a variety of biochemical and biological roles, making them integral to the plant's immune system(Shahidi 1997) (Irnawati, Riyanto et al. 2022) (Preedy 2015).
The extraction and characterization of oil content in Chrozophora tinctoria address the challenge of limited availability of sustainable sources of natural bioactive compounds. While Chrozophora tinctoria is known for its medicinal and industrial uses, research on the chemical composition and properties of the oil extracted from its seeds remains scarce.
By characterizing the chemical composition and bioactivity of this oil, this study aims to contribute to the development of sustainable and environmentally friendly sources of natural compounds with potential health and industrial applications. The oil was extracted using a Clevenger apparatus and characterized using FTIR and UV spectroscopy. The analysis focused on determining the chemical composition and properties of the oil, identifying functional groups, and detecting potential bioactive compounds. IR analysis indicated the presence of amines, ethers, esters, aldehydes, and ketones functional groups.

Material and Method

Sample Collection

The fruit of the Chrozophora tinctoria plant was collected from various regions, specifically within District D.I. Khan's Union Council (UC) Paharpur, including the area of Bader Colony, as well as from Mardan. These locations were chosen for their distinct environmental conditions, which contribute to the natural growth of

Chrozophora tinctoria. The collection process involved identifying healthy plants, ensuring the fruit was harvested at the right stage of maturity, and following local ecological practices to preserve the plant's natural habitat.

Seed's Crushing

After collection, the seeds underwent a cleaning process to remove any impurities or unwanted material. Once thoroughly cleaned, the seeds were carefully crushed into smaller pieces using a mortar and pestle. This manual crushing method was chosen to ensure that the seed structure was broken down evenly, allowing for better extraction and analysis in subsequent stages of the research. By using a mortar and pestle, the process maintained the integrity of the seed components, which is crucial for achieving accurate and consistent results.

Seed's Soaking

The crushed material was transferred into a container weighing 200 mg and methanol 600ml was added to ensure the seed material fully mixed. Mixture was allowed to soak for one day. To dissolve oil components in methanol.

Oil's Extraction

Essential oil's extraction were performed at Botany Department Govt Degree College Paharpur Dikhan and KPK. To extract oil the procedure of Daniella Pingret& Anne-Sylvie Fabiano-Tixier& Farid Chemat(An Improved Ultrasound Clevenger for Extraction of Essential Oils)2013 was followed. The apparatus was assembled, and the soaked seed mixture was transferred into the round-bottom flask of the Clevenger apparatus. Condenser and collection flask were connected to the apparatus. The round-bottom flask was heated using a heating mantle. Methanol evaporated and oil's vapors condensed in the condenser, eventually collected in the Clevenger assembly.

The mixture in the Clevenger assembly was allowed to cool down. The oil and methanol mixture was then transferred into a separator funnel, where it was left to settle, allowing the oil and methanol to separate into distinct layers. The remaining oil was transferred from the separator funnel into a heat-resistant container or beaker. Residual methanol was evaporated from the oil while stirring the mixture on a heating magnetic stirrer. Then oil was stored in an Eppendroff tube. This process was done one time, the collected oil weighed 1.5ml and the same process was repeated the oil was 1ml.

Characterization

FTIR spectroscopy was performed at the Pharmacy Department, Gomal University Dera Ismail Khan KPK. For FTIR spectroscopy the procedure developed by (cebi, arici, and sagdic, 2021) with slight modification was used. Both of the samples were kept in room temperature (25C) for half an hour prior the FTIR spectroscopy. An ATR accessory (single- bounce) was used in all spectral acquisition. Parameters of spectral measurment on the basis of resolution and accumulation were selected as 4cm-1 and 16 scans, respectively. Opus program version 7.2(brukergmbh) was employed for instrument control and data spectroscopy. Samples were placed on a diamond ATR crystal with the help of a Pasteur pipette. . The ATR crystal was sterilized with ethanol (80v\v)prior to each spectral acquisition. The background air spectrum was scanned before each acquisition.

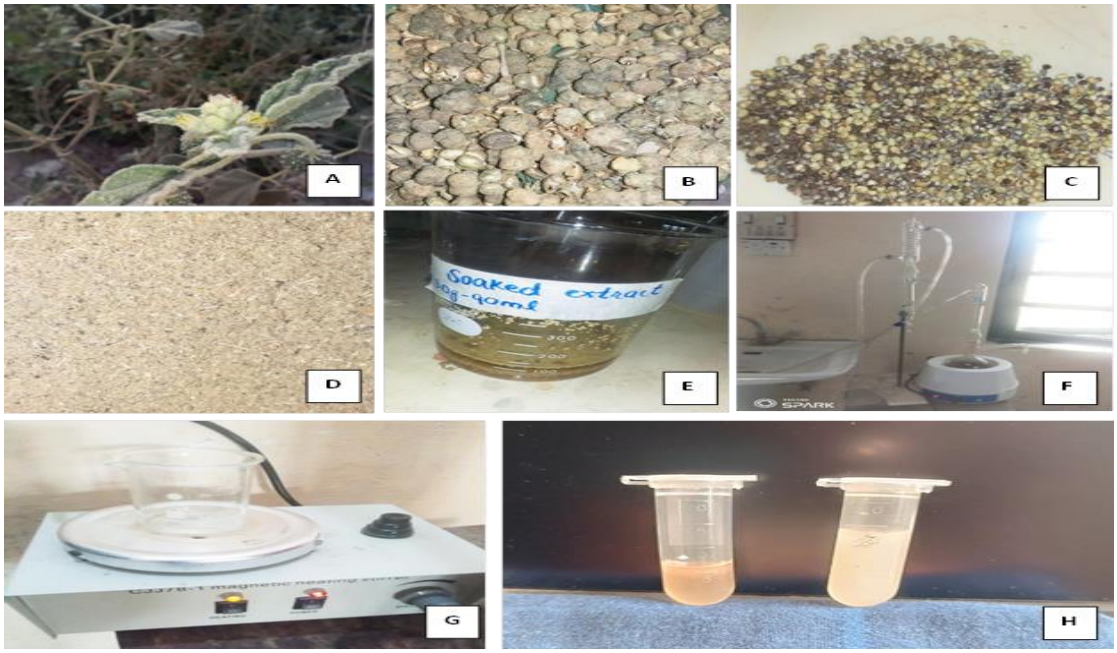


Figure 1(A) *Chrozophora tinctoria* plant (B)crushed plant's fruit (C) cleaned seeds (D) seed's powder (E) seed's extract (F)Clevenger apparatus (G) Heating magnetic stirrer (H) extracted oil.

Results

The oil extraction and characterization of seeds, the extraction process was conducted twice to ensure accuracy and consistency of results. The first extraction yielded 1.5 ml of oil, which appeared yellowish-brown in color, while the second extraction produced 1 ml of oil with off-white. Both samples were carefully monitored, and the entire extraction process spanned duration of seven days. This extended period allowed for a thorough separation of oil from the seeds, ensuring the maximum possible yield. The variation in oil color between the two extractions due to environmental factors actually during first extraction the environment was favorable but during second extraction environmental conditions was little bit harsh, due to this reason colour and concentration variated. Both of the oils sample characterized and showing different functional groups and bioactive compounds which present in them.

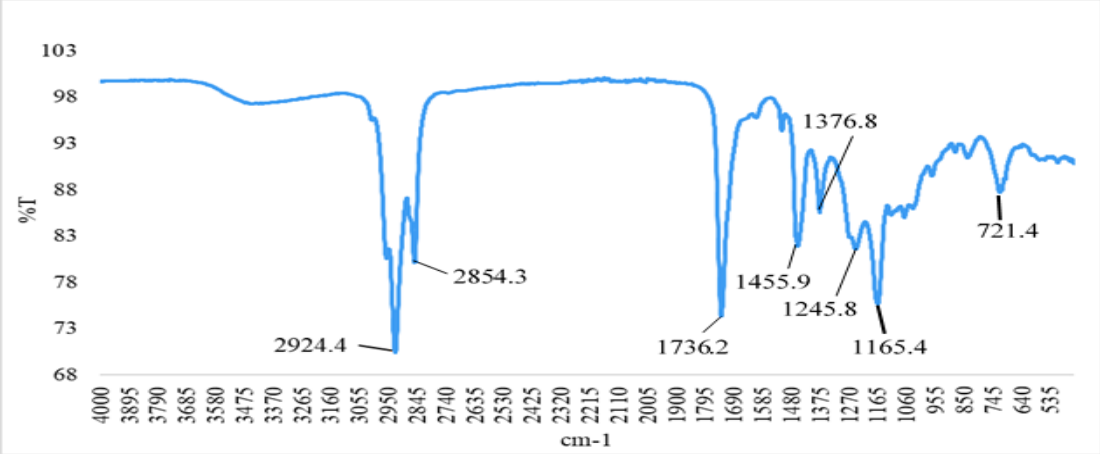
Sample 1;

The sample 1 contains 8 peaks with different values of absorptions as well as transmissions. These peaks in C-H bending indicate the aldehyde, ketoses, and primary or secondary amines, etc as a functional group.721**.** 44cm-1 suggests the presence of an aromatic C-H out-of-plane bending vibration or an out-of-plane bending vibration of a substituted benzene ring. 1165 .38cm-1 corresponds to a C-N stretching vibration, which could indicate the presence of primary or secondary amine groups. 1245. 82cm-1 can be attributed to a C-O stretching vibration, suggesting the presence of an ester or ether functional groups. 1378 .82cm-1 can be associated with C-H bending vibration in an aliphatic structure or methyl groups.

1455 .93cm-1 indicates a C-H bending vibration in an aromatic structure or a methyl group 1736. 16cm-1 corresponds to a C=H stretching vibration, which commonly appears in carbonyl groups such as aldehyde, ketoses, or carboxylic acid.2854.29cm**-1** represents a C-H stretching vibration in an aliphatic structure, typically seen in CH2 or CH3 groups.2924 .35cm-1 indicates a C-H stretching vibration in an aliphatic structure, usually associated with CH2group.

Wave-numbers region (cm^{-1})	Functional groups	Vibration modes
721. 44cm^{-1}	benzene C-H	bending vibration
1165 .38cm^{-1}	amine C-N	stretching vibration
1245. 82cm^{-1}	ester or ether C-O	stretching vibration
1378 .82cm^{-1}	aliphatic, methyl C-H	bending vibration
1455 .93cm^{-1}	aromatic, methyl C-H	bending vibration
1736. 16cm^{-1}	carbonyl groups C=H	stretching vibration
2854.29cm^{-1}	aliphatic (CH2/CH3) C-H	stretching vibration
2924 .35cm^{-1}	aliphatic(CH2) C-H	stretching vibration

Table 1; Functional groups of sample 1 peaks



Graph 1 ; Functional groups of sample 1 peak

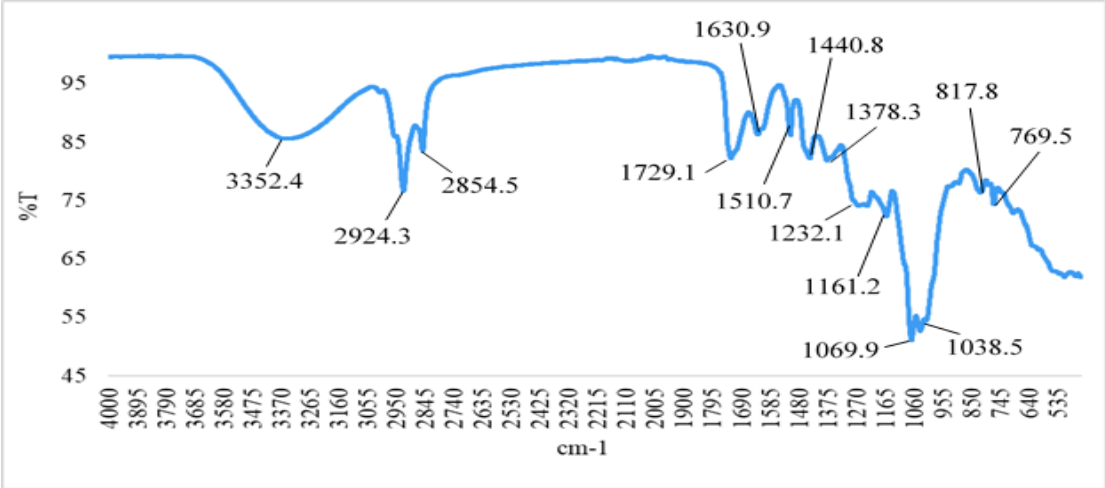
Sample 2

The sample 2 contains 14 peaks with different values of absorption as well as transmission. These peaks in C-H bending represent the bromoalkene, ether, and ester as a functional group. 769.47cm-1 could indicate the presence of a C-Cl stretching vibration, suggesting the presence of a chloroalkane or chloroalkene group. 817.86cm-1 may be associated with a C-Br stretching vibration, indicating the presence of a bromoalkene or bromoalkene group.1038.86cm-1 corresponds to a C-O stretching vibration, suggesting the presence of an alcohol or phenol functional group. 1069.92cm-1 can be attributed to a C-O stretching vibration, indicating the presence of an ether functional group. 1161.23cm-1 suggests the presence of a C-N stretching vibration, which could indicate the presence of an amine group. 1232.02cm-1 corresponds to a C-O stretching vibration, indicating the presence of an ester functional group.

1378.24cm-1 can be associated with a C-H bending vibration in an aliphatic structure or a methyl group. 1440.85cm-1 represents a C-H bending vibration in an aromatic structure or a methyl group. 1510.79cm-1 indicates the presence of an aromatic ring, specifically a C=C stretching vibration in a benzene ring. 1630.97cm-1 corresponds to a C=C stretching vibration in an alkenes group.1729.14cm-1 represents a C=O stretching vibration, which commonly appears in carbonyl groups such as aldehyde, ketoses, or carboxylic acids. 2854.53cm-1 suggests a C-H stretching vibration in an aliphatic structure, typically seen in CH2 or CH3 groups.2924.32cm-1 indicates a C-H stretching vibration in an aliphatic structure, usually associated with CH2 groups. 3352.47cm-1 corresponds to an O-H stretching vibration, typically seen in the hydroxyl (alcohol or phenol) or carboxylic acid functional.

Wave numbers regions(cm^{-1})	Function groups	Vibration modes
769.47cm^{-1}	Chloroalkane/ chloroalkene C-Cl	stretching vibration,
817.86cm^{-1}	Bromoalkene/bromoalkene C-Br	stretching vibration,
1038.86cm^{-1}	alcohol or phenol C-O	stretching vibration,
1069.92cm^{-1}	ether C-O	stretching vibration,
1161.23cm^{-1}	amine C-N	stretching vibration,
1232.02cm^{-1}	ester C-O	stretching vibration,
1378.24cm^{-1}	aliphatic (methyl) C-O	Bending vibration
1440.85cm^{-1}	aromatic (methyl) C-H	Bending vibration
1510.79cm^{-1}	aromatic ring C=C	stretching vibration,
1630.97cm^{-1}	alkenes C=C	stretching vibration,
1729.14cm^{-1}	carbonyl C=O	stretching vibration,
2854.53cm^{-1}	aliphatic (CH2 or CH3) C-H	stretching vibration,
2924.32cm^{-1}	aliphatic (CH2)C-H	stretching vibration,
3352.47cm^{-1}	carboxylic acid O-H	stretching vibration,

Table 2; Functional groups of sample 2 peaks



Graph 2 ; Functional groups of sample 2 peaks

Discussion

The sample 1 contains 8 peaks with different values of absorptions as well as transmissions. These peaks in C-H bending indicate the aldehyde, ketoses, and primary or secondary amines, etc. Sample 2 contains 14 peaks with different values of absorption as well as transmission. These peaks in C-H bending represent the bromoalkene, ether, and ester as a functional group.

The employment of FTIR spectroscopy and chemometrics for the classification and prediction of antioxidant activities of pumpkin seed oil from different origins, the peak at 721cm-1 corresponds to the C-H rocking

vibration, in an alkane structure which is commonly associated with CH2 as a functional Group (Irnawati, Riyanto et al. 2022). This peak is similar to the FTIR analysis of the essential oil of *Chrozophora tinctoria* but shows different results, as in this oil the peak at 721.44cm-1 represents the C-H bending vibration with benzene ring.

Genistein; A new isoflavono from Iraqi *Chrozophora tinctoria*; its extraction, isolation, and structure Elucidation showed, the peak at 2947.23cm-1 represents the C-H stretching due to –CH3.(Abdul-aziz and Abdul-Jalil 2022). At the peak of 1651.07cm-1 showed the presence of C=O, carbonyl group. the peak at 1606.76cm-1 represents the presence of –CH=CH group ,comparatively these peaks are similar to the FTIR analysis of essential oil of *Chrozophora tinctoria* but in this oil the peak at 2924.32cm-1represents the C-H stretching vibration with aliphatic CH2 as a functional group. And at the peak 1630.97cm-1represents the C=H stretching vibration with alkenes as a functional group.

FTIR spectroscopic analysis of functional groups in biodiesel produced from oils of *Ricinus communis,* their results match with results of *Chrozophora tinctoria*. The peak at 732.33cm-1 shows bending vibration with =C-H as a functional group. And the peak at 856.42cm-1 represents bending vibration with =C-H as a functional group. The peak at 1031.95cm1 corresponds to stretching vibration with =C-H as a functional group. The peak at 1199.78cm-1 shows stretching vibration with C-N as a functional group. 1464.02cm-1 represents stretching vibration with C- C as a functional group. The peak at 1743.71 shows stretching vibration with C-O. The peak at 2929.97cm-1 represents stretching vibration with the C- H as a functional group. However, the functional groups of peaks of *Chrozophora tinctoria* show different results.

The peak at 721 44cm-1 suggests the presence of an aromatic C-H out-of-plane bending vibration or an out-of-plane bending vibration of a substituted benzene ring. 817.86cm-1 may be associated with a C-Br stretching vibration, indicating the presence of a bromoalkene or bromoalkene group. 1069.92cm-1 can be attributed to a C-O stretching vibration, indicating the presence of an ether functional group. The peak at 1165 .38cm-1 corresponds to a C-N stretching vibration, which could indicate the presence of primary or secondary amine groups. 1440.85cm-1 represents a C-H bending vibration in an aromatic structure or a methyl group.

FTIR functional group frequencies of Jatropha Biodiesel.2852.81cm-1 show stretching vibration with O-H as a functional group. 2931.9cm-1 indicates stretching vibration with O-H as a functional group. 2854. 53cm-1 suggests a C-H stretching vibration in an aliphatic structure, typically seen in CH2 or CH3 groups. But the functional groups of peaks of *Chrozophora tinctoria* show different results .2924.32cm-1 indicates a C-H stretching vibration in an aliphatic structure, usually associated with CH2 groups.(Ndana, Grace et al. 2013).

Conclusion

The oil extraction characterization of seeds from *Chrozophora tinctoria* (also known as dyer's croton) have been successfully conducted using Fourier transform infrared spectroscopy (FTIR) machine. Oil extraction process involved the separation of oil from the seeds through mechanical pressing or solvent extraction. FTIR analysis provided information about chemical composition of extracted oil. The FTIR spectra revealed characteristic absorption peaks corresponding to various functional groups present in the oil. Such as carbonyl, carboxyl, and alkyl groups. These peaks helped in identifying the types of compounds present in the oil, such as fatty acids, esters, and other lipids components. Furthermore, FTIR analysis allowed for the determination of important oil properties, including the presence of unsaturated fatty acids, oxidative stability, and overall quality assessment. . This knowledge can be utilized for further research, industrial applications, and the development of value-added products derived from this plant species.

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