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Utilizing FTIR Spectroscopy for Phytochemical Assessment of Oregano majorana.

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Abstract

The aim of the current research is to assess the phytochemical composition of Oregano majorana, a species native to the northeastern Punjab region of Pakistan. Oregano majorana. was cultivated in our botanical garden under standard atmospheric conditions. The flowering parts and stems were harvested and stored in darkness for 45 days to prevent any alterations caused by sunlight or artificial indoor lighting. Both samples were then finely ground into powder to analyze the phytochemical profile using Fourier Transform Infrared (FTIR) spectroscopy. FTIR is chosen for its nondestructive nature, cost-effectiveness, and environmental friendliness, offering an ideal platform for researchers. The spectra of the flowers and non-flowering parts exhibited distinct characteristic peaks, allowing for the identification of functional groups present, which were further discussed in correlation with the peaks observed.

1. Introduction

Since the dawn of humanity, botanical resources have served as both nourishment and remedies [1]. Various components of plants, including roots, stems, bark, leaves, flowers, fruits, and seeds, have been utilized in the diets and treatments of humans and animals alike [2]. The phytochemical compositions of plants are influenced by their habitats [3]. These natural compounds found in plants serve as valuable reservoirs of medicinal properties. Herbal medicines, derived from plants, often exhibit a slower yet more enduring efficacy compared to synthetic drugs.

Plants serve as valuable reservoirs of bioactive compounds renowned for their antioxidant, anti-inflammatory, and anticancer properties [4]. The therapeutic efficacy of herbal plants is often ascribed to their rich content of esters, terpenoids, and flavonoids [5]. Extracts derived from herbal plants are widely recognized for their biomedical applications in treating various ailments such as respiratory, gastrointestinal, and dermatological conditions [6]. Oregano majorana L. is renowned for its medicinal, culinary, and herbal uses [7]. Oregano majorana L. contains active compounds known to combat B. subtilis, E. coli, and other Gram-positive and Gram-negative bacteria [8]. Given the medicinal significance of Oregano majorana L., its phytochemical composition was analyzed using FTIR spectroscopy. The oregano plant, along with its flowering twig and dried flowering part, are depicted in Figure 1.

Existing literature highlights the use of plants containing secoiridoids for hepatic diseases through FTIR analysis [9]. Various bioactive compounds found in the leaves of Calotropis gigantea have been thoroughly investigated [10]. Indian plants have been screened for the presence of saponins, flavonoids, and phenolic compounds [11]. The leaves, stems, and roots of Eclipta alba are recognized as rich sources of bioactive compounds [12]. Similarly, plants such as Mentha spicata, Aloe Vera, Croton, Portulaca, Aervalanata, Ichnocarpus frutescens, and Ampelocissus latifolia are widely acknowledged for their medicinal properties [13-19].

The present study aims to assess the phytochemical content of Oregano majorana L. using FTIR spectroscopy with KBr pellet technique on a Varian 640-IR instrument. FTIR spectroscopy has gained popularity in research due to its nondestructive nature in analyzing biological specimens [20]. The fingerprint region of spectral lines (600-1450 cm^-1) holds particular significance for cross-referencing biological specimens [21].

2. Resources and Methodology

Identified by its leaf morphology and inflorescence structure, the plant is identified as Oregano majorana L. It was sourced from the suburban area of Rawalpindi, where it grows wild. In efforts to ensure its conservation, a few specimens were carefully collected from the wild and cultivated in an herb garden for further research.

During the month of August, the elongated flowering bodies (inflorescences) were harvested and separated from the main plant for drying. After shade drying, the flowering heads were manually isolated from the main floral body. Both the flowering heads and the remaining plant parts were then ground into a powder and subjected to analysis. The analysis was conducted using the KBr pellet method with Varian 640 IR FTIR spectroscopy, scanning within the range of 4,000 – 400 cm^-1."

In the start of March, white flowers were again grown on *Oregano majorana* L and flowers were plucked and separated successfully from green part (head) for further study as shown in Fig. 2.

Fig. 1. *Oregano majorana* L and flowering parts

Fig. 2. White flowers of *Oregano majorana* L.

3. RESULTS AND DISCUSSION

The FTIR spectra of both the flowers and the non-flowering section of the inflorescence are depicted in Figures 3 and 4, respectively. Table 1 summarizes the significant absorption lines observed in both samples. Interestingly, the FTIR spectra of the flowering and non-flowering sections exhibited striking similarity, which contrasts with our previous observations in other plant species [13]. This discrepancy may be attributed to the relatively minute size of the flowers compared to the green, non-flowering portion. Thus, it is plausible to assume that the presence of phytochemicals in the green, non-flowering portion has significantly contributed to the signals observed in the corresponding FTIR spectra.

Based on the FTIR spectra, the expected phytochemical classes of compounds are listed in Table 1. Both spectra confirm the absence of moisture, as evidenced by the lack of a strong absorption band in the spectral region between 3400 cm^-1 and 3100 cm^-1, indicating the absence of hydrogen-bonded OH [22]. The IR spectra of the powdered flowers (Figure 3) and non-flowering part (Figure 4) were found to be similar to each other, likely due to the reasons discussed above.

Collectively, it can be inferred that peaks observed at 1000 cm^-1 are characteristic of alkene compounds (such as Carvacrol, Caryophyllene, 1,3-pentadiene, resorcinol, terpine, linalool, etc.) [23]. Peaks at 820 cm^-1 are also associated with R2C-CHR (alpha-terpineol, pinene, etc.) type compounds [24]. Peaks at higher frequency values, such as 1250 cm^-1, correspond to alcohols and proline, whereas lower peak values around 1235 cm^-1 are indicative of acids and ethers (R=C-O-C) [25].

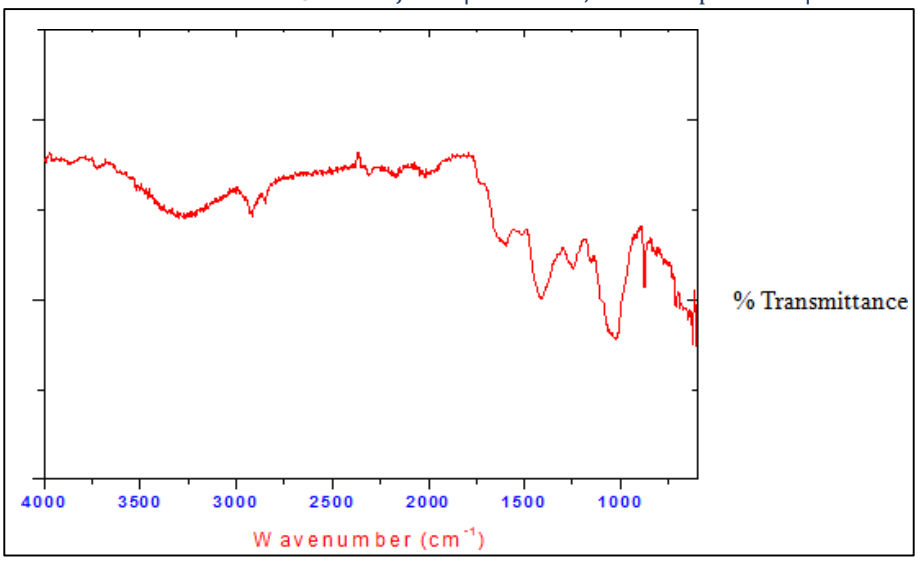

Fig. 3. FTIR spectrum of flowers of *Oregano majorana* L.

Table 1. On the basis of FTIR spectra, expected phytochemical class of compounds are tabulated

Sr. no.	Wave No. cm^{-1} (Test sample)	Wave No. cm^{-1} (Reference)	Appearance	Group assigned	Expected phytochemical class
1.	2937	3200-2700 3000-2800	Weak broad strong	O-H stretching N-H stretching	Alcohols Amine salts
2.	2875	3000-2840	medium	C-H stretching	Alkanes
3.	1625	1650-1600	medium	C=C stretching	Conjugated alkenes
4.	1416	1420-1330	medium	O-H bending	Alcohols
5.	1235	1250-1020	medium	C-N stretching	Amines
6.	1250	1275-1200	strong	C-O stretching	Aromatic esters
7.	1000	650-1000	strong	C=C bending	Alkenes
8.	829	840-790	strong	C=C bending	Alkenes

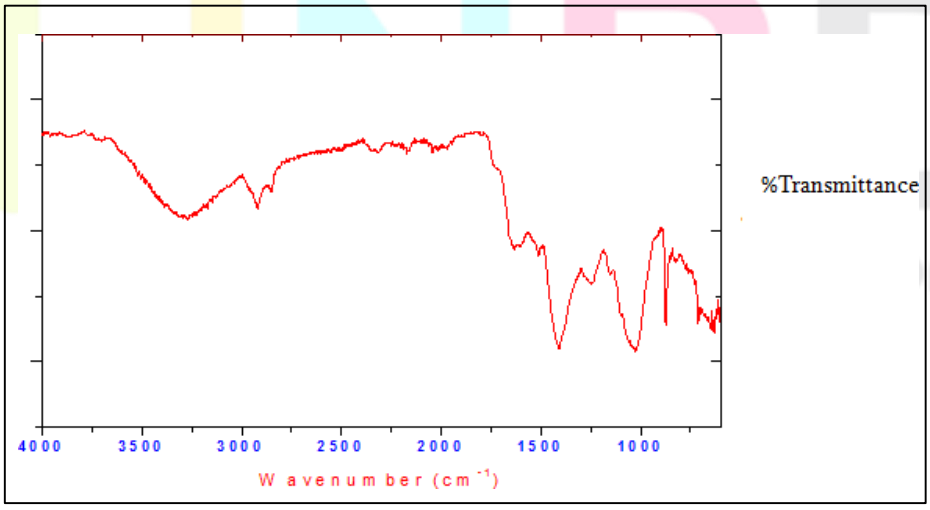

Fig. 4. FTIR spectrum of non-flowering part of flowering twig of *Oregano majorana* L.

Peaks near 1416 cm^{-1} are related to saturated alkyls, Peaks at 1625 cm^{-1} shows the presence of C=C belonging to aromatic compounds. FTIR of both flowering and non-flowering twig have shown broad signal of medium intensity in the region between 3600 cm^{-1} and 3100 cm^{-1} that corresponds to the presence of non-hydrogen

bonded OH, along with weak but characteristic signals of small intensity between 2937 cm^{-1} and 2875 cm^{-1} specific to sp^3-C-H stretch indicating bicyclic, phenolic and many other classes of compounds.

4. Conclusion

The plant species within the Croton genus are recognized for their abundance of significant phytochemicals, primarily falling into the category of cyclic terpenoids, notably di-terpenoids such as monocyclic and aliphatic aromatic compounds, alongside organic phenols including gallic acid, caffeic acid, vanillic acid, and syringic acid. The distinct FTIR spectra obtained from both the flowers and non-flowering portions demonstrate that finely powdered samples of delicate plant parts can be accurately analyzed using this technique, provided they are thoroughly shade dried and devoid of moisture. The presence of active functional groups closely aligns with the observed signals, particularly those associated with non-hydrogen bonded –OH groups, saturated hydrocarbon functionalities, and alkenes, among others. This study underscores the medicinal significance of the plant and proposes avenues for further advancement in spectroscopic techniques to elucidate the structural composition of the compounds found in oregano.

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