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Evaluation of Herbal Samples Constituents by Applying Phytochemical Screening Approach and Fourier Transform Infrared Spectroscopy

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ABSTRACT

Herbal medicines are continuously contributing a significant role in the prophylaxis and healing of diverse ailments worldwide. When analyzing the chemical constitution of herbal medicines, the important challenge is their complex chemical composition. Fourier transform spectroscopy and phytochemical screening are two significant analytical tools to determine the chemical constitution of a sample. The present research work is focused on comparing the data obtained by these two techniques to assess the type of constituents present in herbal samples. *Cichorium intybus* and *Lepidium sativum* seeds were procured from reliable herbal shops in Karachi and were identified, authenticated, powdered, extracted, and then subjected to the standard procedures of qualitative phytochemical screening and Fourier transform infrared spectroscopy. Results obtained through these two analyses were compared and interpreted. Diverse peak intensities at different wavelengths have been observed in Fourier transform infrared (FT-IR) spectra of two herbal samples. Leading functional groups observed include carboxylic acids, phenols, aromatics, alkanes, alkenes, alkynes, alcohol, amines, amides, sulfate esters, ethers and alkyl halides while lipids, flavonoids, carbohydrates, proteins, alkaloids, triterpenes, tannins, phlobatannins, anthraquinones, saponins, and quinones were major primary and secondary metabolites detected in phytochemical screening. FTIR is an effective technique for the determination of the structure and composition of a herbal sample based on the vibrational properties of functional groups, whereas phytochemical screening is the primary and qualitative technique to detect primary and secondary metabolites in herbal samples. Comparison of results attained by these two techniques helps identify constituents and their intensities in different herbal samples.

INTRODUCTION

Bioactive constituents obtained from natural sources have been examined for their specific properties and medicinal effects. Plants are vast sources of natural bioactive components in the form of secondary metabolites [1]. In the current era, a vast interest is developed in bioactive compounds derived from natural sources, also known as secondary metabolites, including alkaloids, flavonoids, coumarins, tannins, glycosides, phytosterols, etc., with huge medicinal effects, e.g., antioxidant, anticancer, anti-inflammatory, antibacterial, antiviral, and immunomodulation properties [2]. Fourier transform infrared spectroscopy (FT-IR) plays a significant role in the identification of these bioactive constituents, as the vibrational spectrum is considered a characteristic property of each molecule. Infrared spectra can be utilized as a fingerprint, and by comparison of unknown spectra with reference spectra, constituents present in a sample may be identified. The more practical approach is that the visual aspect of spectroscopy is very important to determine the chemistry and structure of a sample, and this is accomplished by identifying specific patterns as well as shapes inside the spectrum with the application of published group frequency information, together with chemical and physical data of the sample. In addition, the infrared spectrum is greater than simply assigning only the group frequencies, as this is abundant in information [3]. Infrared spectroscopy explores molecular vibration. Specific infrared absorption bands are associated with characteristic functional groups related to the basic vibration of functional groups [4]. Phytochemical screening is another significant analytical tool to attain the

preliminary chemical data of a sample and has been used to characterize the main constituents present in a sample. Phytochemical constituents that exist in plants are classified as primary and secondary metabolites. Secondary metabolites act as therapeutic agents for the treatment of different diseases, also known as phytochemicals, and contribute to plant defense against different microbial infections and pest infestation. These phytochemicals may serve as precursors for semisynthetic drugs and play a significant role in drug discovery. Phytochemical screening not only explores the constituents present in natural extracts but also plays a big role in searching for medicinal bioactive constituents responsible for therapeutic effects [5, 6]. Phytochemical screening also plays an important part in drug discovery as it is a method of evaluating, extracting, experimenting, and identifying diverse classes of constituents present in a herbal sample or plant extract [7]. In phytochemical screening, organic and aqueous extracts of plants are subjected to different standard tests mentioned in the literature to identify the presence of diverse secondary metabolites like tannins, alkaloids, flavonoids, terpenes, and other phytoconstituents present in plants. This is a simple technique, after which extracts may be subjected to other separating techniques like thin-layer chromatography to explore the identification and isolation of different components [8].

The present research work aims to compare the FT-IR spectrum with phytochemical screening results to evaluate the main constituents present in herbal extracts. Peak intensities at particular wavelengths reveal the specific functional groups and their intensities in comparison with phytochemical screening results to confirm a specific type of constituents and their concentrations in herbal samples. This gives an initial idea regarding the constituents present in the herbal sample and then goes towards more advanced techniques to further isolate and purify the main bioactive constituents responsible for therapeutic effects.

METHODOLOGY

Cichorium intybus seeds and *Lepidium sativum seeds* were included in this study and procured from a reliable herbal shop in Karachi, Pakistan. The Pakistani population frequently utilizes the selected herbal samples for the cure and management of different ailments. Samples were appropriately stored in air-tight, moisture-free amber-colored bottles.

Identification and Coding of Samples: *Cichorium intybus* seeds (Kasni/Chicory) and *Lepidium sativum* seeds (Garden Cress/Hab Rchad) were in crude form. They were identified, coded as KNI and HRD, and authenticated by Dr. Mohtasheem ul Hasan, Professor, Department of Pharmacognosy, University of Karachi.

Preparation of Powder and Extracts: The samples were initially cleansed, subsequently pulverized by an electrical grinder till powdered. For ethanolic extraction, 50g of seeds were soaked in ethanol for 10 days and then condensed using a rotary evaporator [9].

Phytochemical Qualitative Analysis: All the reagents used were of analytical grade, extracts were evaluated for the presence of Alkaloids (Dragendorff's reagent and Wagner's reagent) [10], Proteins (Biuret test and Ninhydrin test) [11, 12], Triterpenoids (Salkowski test) [11], Carbohydrates (Molisch's and Fehling tests) [13], Flavonoids (Sulfuric acid and lead acetate tests) [14], Saponins (foam tests) [14], Tannins (Ferric chloride test) [10, 14], Phlobatannins [15], Anthraquinones (Modified Borntrager's test) [16], Quinones [14] and lipids [11].

Assessment of Functional Groups by FT-IR: Bruker's Tensor II ATR-FTIR apparatus, accompanied by OPUS 7.5 software, was used for the spectrum generation of studied samples between 4000/cm to 400/cm wave number [17]. After getting spectra of herbal samples, they were interpreted for possible functional groups, and intensities at specific wavelengths using IRPal 2.00 software and literature.

RESULTS

Phytochemical Qualitative Analysis

The present phytochemical assessment focused on the detection of primary and secondary metabolites. The results of phytochemical screening are presented in Table 1.

Table 1

Phytochemical Analysis of Cichorium intybus and Lepidium sativum Seeds

Identification Tests		*Cichorium intybus* (KNI)	*Lepidium sativum* (HRD)
Tests for Alkaloids	Dragendorff's test	++	++
	Wagner's test	++	++
Tests for Proteins	Biuret test	+	++
	Ninhydrin test	++	+++
	Test for Triterpenes/ Steroids	+	++
Tests for Carbohydrates	Molisch's test	++	++
	Fehling's test	-	-
Tests for Flavonoids	Sulfuric acid test	+	+
	Lead acetate test	+	+
	Test for Saponins	+	+
Tests for Tannins	Ferric chloride test	++	++
	Phlobatannins test	-	+
Test for Anthraquinones		++	+
Test for Quinones		+	+
Test for Lipids (Fixed oil/fats)		+++	+++

Where (+) = Low Concentration, (++) = Moderate Concentration, (+++) = Maximum Concentration, (-) = Absent

Cichorium intybus seeds phytochemical screening results displayed a maximum concentration of lipids, and moderate concentration of alkaloids, proteins, tannins, and anthraquinones, whereas a small concentration of triterpenes, saponins, flavonoids, and carbohydrates.

Lepidium sativum seeds exhibited a high concentration of proteins, fixed oil/fats, moderate concentration of alkaloids, tannins, carbohydrates, and triterpenes, whereas a small concentration of flavonoids, saponins, phlobatannins, quinones, and anthraquinones.

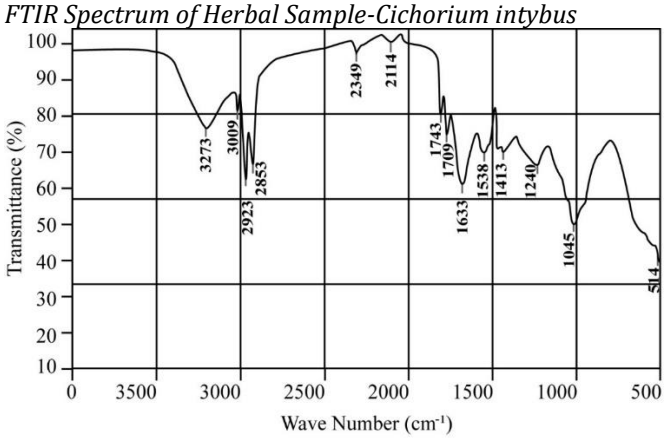
Assessment of Functional Groups by FTIR

The FT-IR spectrum of *Cichorium intybus* seeds as presented in Figure 1 showed medium peaks at 2923 cm^{-1}, 2853 cm^{-1}, 1633 cm^{-1}, 1538 cm^{-1}, 1413 cm^{-1}, 1240 cm^{-1}, 1045 cm^{-1} and 514 cm^{-1} which displayed the potential presence of phenols, carboxylic acid, alkynes, alkanes, amides, amines, sulfate esters, aromatics, alcohol, ethers

and alkyl halides functional groups.

Figure 1

FTIR Spectrum of Herbal Sample-Cichorium intybus



The FTIR spectrum of *Lepidium sativum* seeds as shown in Figure 2, depicted strong peaks at 1040 cm^{-1} and 580 cm^{-1} which showed the probable presence of carboxylic acids, esters, sulfoxides, alkyl halides, and amines functional groups while medium peaks at 3300 cm^{-1}, 2910 cm^{-1}, 2840 cm^{-1}, 1740 cm^{-1},1660 cm^{-1}, 1580 cm^{-1}, 1490 cm^{-1}, 1130 cm^{-1} and 800 cm^{-1} which showed the potential presence of phenols, carboxylic acid, alkynes, alkanes, ketones, esters, amines, amides, alkenes, aromatics, alky halides and imines functional groups.

Figure 2

FTIR Analysis of Herbal Sample-Lepidium sativum

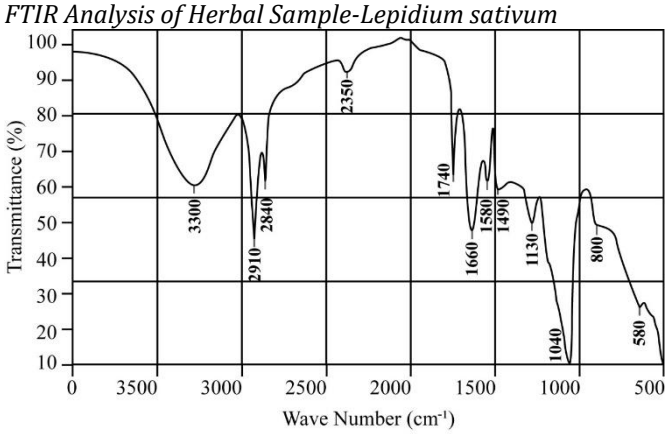


Table 2

Some Important Functional Groups with the Specific Wavelength and Stretching

Functional group	Wavelength	Type of Stretching	Reference
Carboxylic acid	2400–3600 cm^{-1}	O-H	[24]
Ester	1710 cm^{-1}	C=O	
Phenol	3200-3700 cm^{-1}	O-H	[22]
	1040-1210 cm^{-1}	C-O	
Amides	3100-3500 cm^{-1},	N-H	
Amines	3300-3500 cm^{-1}	N-H	
Aromatics	3000-3040 cm^{-1}	C-H	
	1400-1620 cm^{-1}	C=C	
Quinones	1300 cm^{-1}-1700 cm^{-1}	C=O	[30, 31]
Ether linkage Alcohol groups	3402 cm^{-1} and 3318 cm^{-1}	O-H broad	[26]
	1200 cm^{-1} and 1000 cm^{-1}	C-O	
Alkenes	3100-2800 cm^{-1}	C-H	[37]
	1680-1640 cm^{-1}	C=C	
Alkyl Halides	850-550 cm^{-1}	C-Cl	[38]
	690-515 cm^{-1}	C-Br	

DISCUSSION

Organic compounds produced by plants that help in the essential growth and development of plants are known as primary metabolites, whereas the compounds derived from these primary metabolites and contribute to plant defense, metabolism, and other activities are known as secondary metabolites and are related to diverse therapeutic effects [18]. This research work is focused on the problem that is encountered due to the complicated constitution of a herbal sample, as different primary and secondary plant metabolites are found in herbal extract and formulation, and by using phytochemical screening results and their comparison with the FTIR spectrum, utilizing different functional groups and their intensities can make the process less complicated.

Fourier transform infrared spectroscopy (FTIR) is a precise method for identification and demonstration, and has been widely employed in diverse fields. FTIR has been applied in the discipline of medicine not only for diagnosis and analysis but also for finding new medicines [19]. FTIR has been used for the detection of the structure and composition of samples in academia as well as industry [20]. FTIR is a feasible method that is used for the study of powders, liquids, pastes, solutions, films, gases, and fibers. Material that is usually presented on the exterior of the substrate is also analyzed by FT-IR [21].

Prominent functional groups usually observed in a spectrum include carboxylic acid, amide, ester, cyanide/nitrile, amine, ketone, alcohol, aldehyde, aromatics, alkane, alkyne, alkene, nitro, and ether, whereas important bonds usually detected in a spectrum are C-H, O-H, C=O, C=C, C≡C, N-H, C≡N, C-N, C-O, N-O. C-H bonds are found in alkanes, alkenes, alkynes, and aromatics. O-H bonds observed in phenols, alcohols, and acids, C=O bonds in aldehydes, esters, ketones, amides, and acids, C=C in alkenes and aromatics, N-H in amines and amides, C-N in amines, amides, alkyl, and aryl, while C-O in alcohols, phenol, and acids [22].

One of the key constituents of lipids is carboxylic acid [23]. Usually, it is observed that absorption of O-H stretching of carboxylic acid is seen at a broad level, i.e., 2400–3600 cm^{-1} whereas absorption of C=O stretching of the carboxylic acid is generally detected at near 1710 cm^{-1}. The occurrence of carboxylic acid is identified by wide absorption at either 3000 cm^{-1} and 1700 cm^{-1} regions. Amino acid, which is the basic component of protein, also possesses a carboxylic acid. Distinct carboxylic acid derivatives include amides, esters, acyl halides, and acid anhydrides [24]. Alkynes are also part of lipids [25]. O-H stretching of phenol is generally detected at 3200-3700 cm^{-1}, N-H stretching of amides at 3100-3500 cm^{-1}, and amines at 3300-3500 cm^{-1}. Aromatics C-H stretching is usually observed at 3000-3040 cm^{-1}, while C=C stretching is at 1400-1620 cm^{-1}. C-O stretching of phenols is frequently detected at 1040-1210 cm^{-1}[22]. Carbohydrates take part widely in hydrogen bonding and strong stretches of O-H broad observed between 3402 cm^{-1} and 3318 cm^{-1}. Numerous peaks of C-O stretches were found at the region between 1200 cm^{-1} and 1000 cm^{-1}. Carbohydrates structurally consist of an ether linkage and several alcohol groups [26]. Flavonoids and tannins are categorized as subgroups of phenolic

substances [27]. Anthraquinones are compounds that also possess phenolic nuclei [28]. Ester's existence describes the dominant presence of lipids as well as essential oils [29]. Quinone compounds are more prominently observed at 1300 cm^{-1}-1700 cm^{-1} with more distinct C=O stretching vibrations [30, 31]. Essential oil is an important plant constituent composed of alkenes [32]. Though chlorine is present abundantly in plants, especially wood halogen compounds are found in lesser amounts as compared to other constituents like alkaloids, glycosides, tannins, carbohydrates, proteins, etc. Numerous significant organochlorines, as well as other halogen compounds, have been found in different parts of plants. Marine organisms are rich sources of organohalogens, including chlorine and bromine [33, 34]. Different functional groups and their wavelength are presented in tabular form in Table 2.

In the present research work, the herbal sample *Cichorium intybus* FTIR spectrum, as presented in Figure 1, showed a medium presence of carboxylic acids, aromatics, alcohol, alkanes, alkynes, amides, amines, sulfate esters, ethers, and alkyl halides functional groups. Phytochemical analysis of this herbal sample as presented in Table 1, revealed a high concentration of fixed oil and a reasonable concentration of proteins, alkaloids, tannins, and anthraquinones, while minute concentrations of saponins, triterpenes, carbohydrates, and flavonoids were also detected. A comparison of two studies showed that carboxylic acids, alkanes, alkynes, and sulfate esters confirm the high concentration of lipids. amides and amines indicated the presence of proteins and alkaloids. Ether and alcohol functional groups ratified the presence of carbohydrates. carboxylic acid and sulfate esters demonstrated the presence of triterpenes and saponins. Aromatics verified the presence of flavonoids, tannins, and anthraquinones. Fluorinated and brominated compounds were also observed. This is because chlorine is present abundantly in plants, especially wood, but halogen compounds are found less as compared to other constituents like alkaloids, glycosides, tannins, carbohydrates, proteins, etc. Numerous significant organochlorines, as well as other halogen compounds, have been found in different parts of plants. Marine organisms are rich sources of organohalogens, including chlorine and bromine. Literature also reported that *Cichorium intybus* contains a high concentration of crude protein including essential amino acids, plentiful seed oil both saturated and unsaturated fatty acids, inulin, and a rich source of minerals, carbohydrates, flavonoids, tannins, sesquiterpene lactones, phenolic compounds, coumarins and found hepatoprotective [35, 36].

In sample, *Lepidium sativum* the FTIR spectrum, as shown in Figure-2, depicts strong peaks at 1040 cm^{-1} and 580 cm^{-1} which showed the probable presence of carboxylic acids, esters, sulfoxides, alkyl halides, and amines functional groups while medium peaks at 3300 cm^{-1}, 2910 cm^{-1}, 2840 cm^{-1}, 1740 cm^{-1},1660 cm^{-1}, 1580 cm^{-1}, 1490 cm^{-1}, 1130 cm^{-1} and 800 cm^{-1} which showed the potential presence of phenols, carboxylic acid, alkynes, alkanes, ketones, esters, amines, amides, alkenes, aromatics, alky halides and imines functional groups. Phytochemical screening results, as shown in Table 1, showed that *Lepidium sativum* exhibited a high concentration of proteins, fixed oil/fats, and a moderate concentration of alkaloids, tannins, carbohydrates, and triterpenes, whereas a small concentration of flavonoids, saponins, phlobatannins, quinones, and anthraquinones. The comparison of both results verified the maximum existence of proteins and fixed oil as carboxylic acid, esters, alkynes, alkanes, and alkenes functional groups indicated the presence of lipids, whereas amines, amides, and imines proved the high concentration of proteins as well as alkaloids. Phenols and aromatics evidenced the existence of flavonoids, tannins, phlobatannins, anthraquinones, and quinones. The carboxylic acids and esters functional groups described the presence of triterpenes and saponins. C-O stretching at 1130 cm−1 -1200 cm−1 evidenced the presence of carbohydrates. The potential existence of brominated, fluorinated, and chlorinated compounds is possible.

This is the simple method that can be used to analyze the type of constituents before going to the more advanced techniques in any herbal sample, whether in crude form or finished product.

CONCLUSION

Phytochemical screening is the preliminary tool to detect primary and secondary metabolites in herbal samples. It is an efficient qualitative test for the identification of particular constituents, whereas FTIR is an effective technique for the determination of structure as well as composition of a herbal sample based on vibrational properties of functional groups present in a sample and used for identification and quantification. The comparison of both studies gives an idea and understanding of the chemical composition of herbal samples from an analytical point of view and for research purposes. It also provides an idea about some impurities that may be present in a sample. In most of the understudied herbal sample flavonoids, carbohydrates, lipids, tannins, alkaloids, proteins, anthraquinones, triterpenes, and alkaloids were detected. Prominent functional groups observed include phenols, carboxylic acids, esters, amines, amides, alkenes, ether, aromatics, alcohol, alkanes, alkynes, and alkyl halides.

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