

EXTRACTION OF PIPERINE AND SPECTRAL ANALYSIS USING IR AND UV VISIBLE SPECTRAL DATA IN SELECTED SOLVENTS

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Abstract- Piperine is a pungent alkaloid present in black pepper (*Piper nigrum*) and other species of the Piperaceae family. It has various biological activities, such as anti-inflammatory, anticancer, antiviral, and bioenhancer. The extraction of piperine from pepper can be done by different methods, such as solvent extraction, supercritical fluid extraction, microwave-assisted extraction, and enzyme-assisted extraction. The spectral analysis of piperine can be performed by infrared (IR) and ultraviolet-visible (UV-Vis) spectroscopy, which can provide information about the functional groups and electronic transitions of the molecule. In this study, we extracted piperine from black pepper using different solvents and analysed its IR and UV-Vis spectra in selected solvents. We compared the extraction yield, purity, and spectral characteristics of piperine in different solvents the solvents had different effects on the IR and UV-Vis spectra of piperine, depending on their polarity and hydrogen bonding ability. Our study provides a comprehensive review of extraction and spectral analysis of piperine from black pepper.

Keywords– Piperine, Black pepper, Infrared (IR), UV Visible (UV), UV-Vis Spectra, Spectroscopy/spectral analysis, Extraction.

I. INTRODUCTION

There are 4 isomers of Piperine mainly as geometrical isomers [1] The other forms are also identified. There is light induced conversion and isomerisation to form other forms [2]. Black pepper bears a great medicinal, economic, and commercial potential [3].

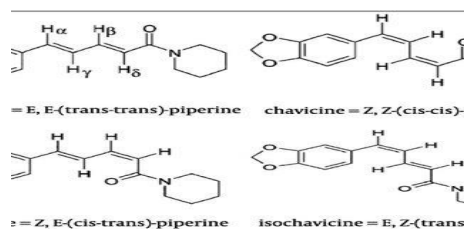


Fig.1: Structure of piperine

II. MATERIALS AND METHODS

At First, 100 grams of black pepper was brought from local general store and was finely ground using mixer grinder and stored separately. Then, 25 g of finely powered black pepper was weighed and it was added with 300 ml of 95% ethanol and was kept in reflux condenser for 1 hour. Pepper solution was then filtered using Whatman's filter paper. The clear filtrate was then kept for boiling until the volume of the filtrate was concentrated to about 25 ml by distillation on a water bath. To the residue, 25 ml of 2N KOH was added and solution was warmed and filtered. The filtrate is heated in steam bath and water was added drop wise till the turbidity appears. The turbid solution was refrigerated overnight for further crystallization process. The refrigerated solution was further filtered using acetone. After filtration, fine yellow-coloured needles were obtained. The yellow-coloured needles were used to determine the melting point. The Melting Point was determined using the standard procedure, the obtained melting point is 127 °C. The piperine extract (yellow-coloured needles) was baked using oven to remove water content (dehydrate the product) and made it completely crystalline, from Black pepper seeds ethanol solvent extraction with KOH gave a good yield having insoluble material [5].

25g of Black pepper powder
↓ treated with
300 mL of Ethanol
(1 hour Reflux condenser extractor)
↓ Hot filtration
300 mL to 25 mL of extract solution on water bath
↓ 25 mL of 2N KOH

Water was added dropwise till the turbidity appeared
 ↓ Kept in refrigerator overnight
The refrigerated solution was further filtered using acetone
 ↓
The fine yellow crystals appeared

Chromatography

Silica Gel TLC was employed to identify the purity of piperine obtained the mobile phase consisted of 9:1 chloroform and acetone mixture. The spraying agent employed was Iodine Vapours that produce pink coloured spots [Fig.2].

The R_f values for the given solvent was calculated as 0.454 and it was found to be within the range of given R_f value for piperine. The crystalline piperine thus obtained in pure state is subjected to UV Vis spectrophotometer and IR spectroscopy.



Fig.2: TLC sheet in silica gel



Fig.3: TLC result

The Thin Layer Chromatography was performed in which the extracted Piperine was taken and was crystallized with ethanol, the TLC was performed using Silica gel. The R_f value was calculated after TLC. The R_f value was obtained well within the range according to the literature [4]

R_f Value Calculation:

Length of the solvent run up from the marked point: 5.5

Length of the spot from the marked point: 2.5

$$\text{Retardation factor (R}_f\text{)} = \frac{2.5}{5.5} = 0.454$$

IR Spectra

Infrared Spectroscopy was carried out by Attenuated Total Reflectance (ATR) to perform the spectral analysis of the obtained Piperine crystals. IR was performed using Alpha E IR machine from Bruker Company. ATR-IR was chosen over FTIR because it has a slight advantage like ATR is a quick & flexible technique and ATR can also provide highly reproducible results for a wide variety of sample types such as threads, yarns, fabrics, fibers, pastes, powders, suspensions, polymers and rubbers. Which may otherwise be difficult and/or time consuming to sample using a transmission technique. Minimal sample is sufficient for ATR. Just placing the sample on the crystal can collect the data, after running the IR Crystals can be cleaned very quick and easily. ATR is a type of IR technique where the obtained solid recrystallized sample is placed in the Infrared Spectrophotometer Crystal and is run and the data is extracted with the help of computer.

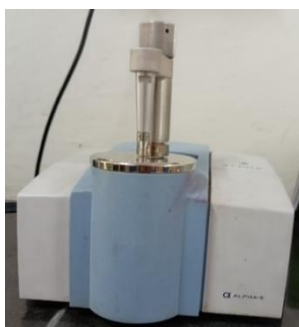


Fig.4: Bruker Alpha II ATR-IR Spectrophotometer

III.RESULTS AND DISCUSSIONS

The interpretation of graphical data is compared with KBr disc and CCl₄ solvent IR standard as given below [6].

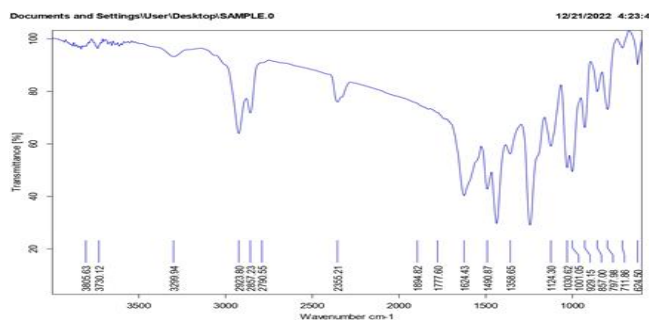


Fig.5: IR results

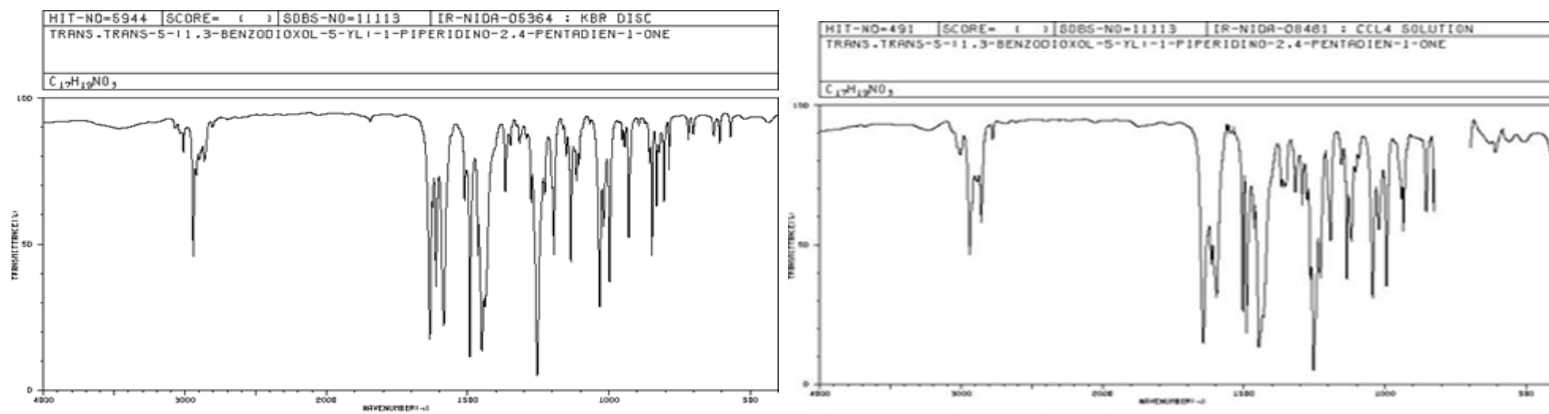


Fig.6: IR graphs Results obtained for KBr disc and CCl₄ solvents respectively referred for the IR analysis

UV Vis Spectra

Ultraviolet - Visible (UV - Visible) Spectrophotometry is primarily quantitative Analytical technique concerned with the absorption of near UV (150-400nm) or Visible (390-780 nm) radiation by chemical species in solution. UV- Visible Spectrophotometry is a quantitative technique used to measure how much a Chemical substance absorbs light. It was developed by Arnold Beckman in July 1941. Spectrophotometric method is usually less sensitive, which Accommodate concentration of few Nano moles per ml. This technique enables the determination of micro molar concentrations of substances and has a broad scope of applications in this field since most biochemical compounds absorb in the UV - Visible region or can be converted into absorbance derivative.

Preparation of standard solution:

The piperine obtained was used to prepare stock solution in 4 different solvents namely Ethanol Methanol Acetone and Carbon tetra chloride

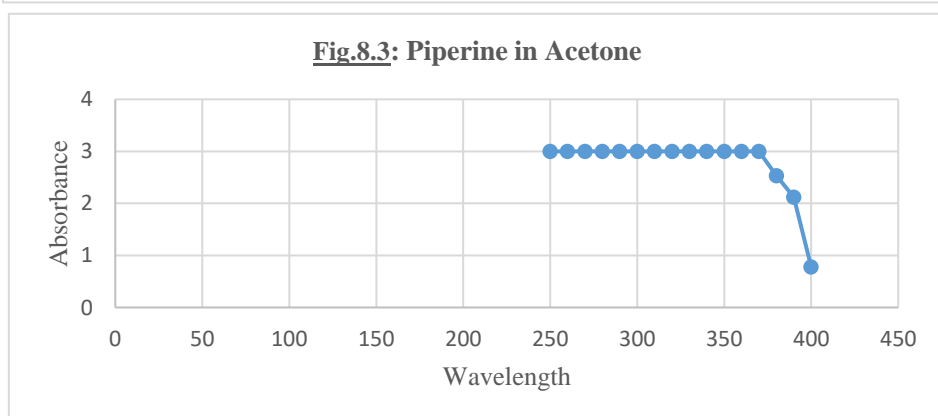
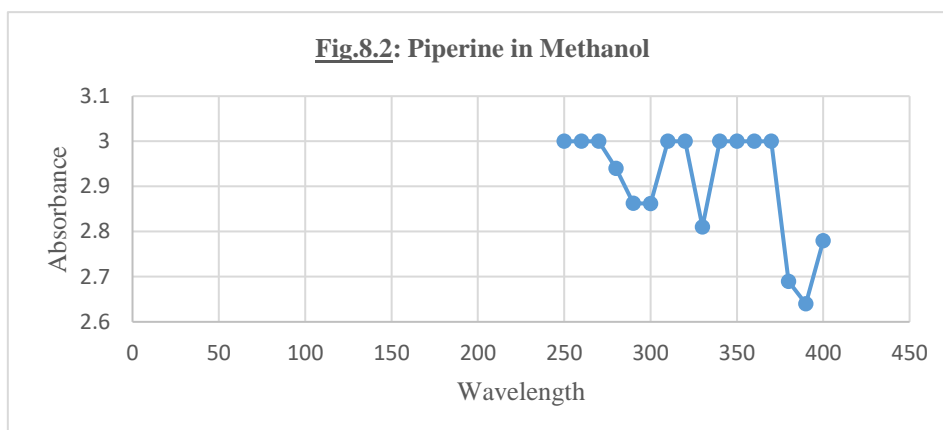
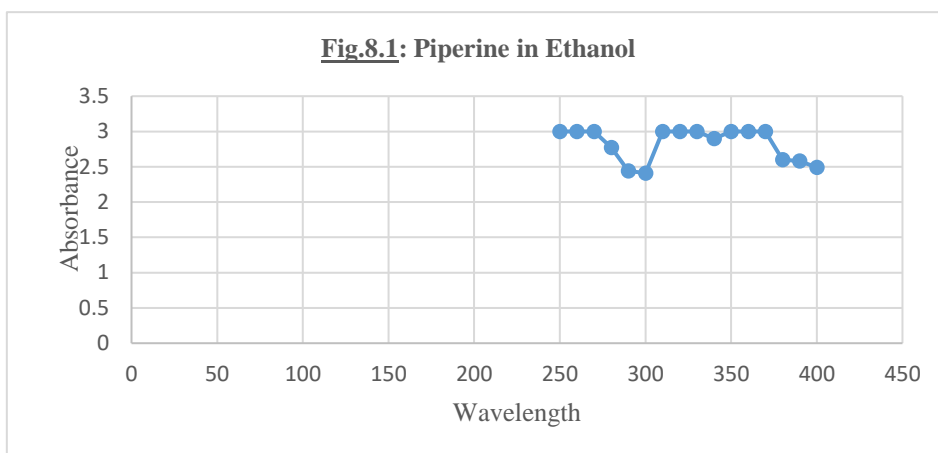
The concentration range for these solvents used from stock solution was found to be 40Mg per ml for all solvents. The calibration curve was developed at a wavelength range between 250 to 400 in common.

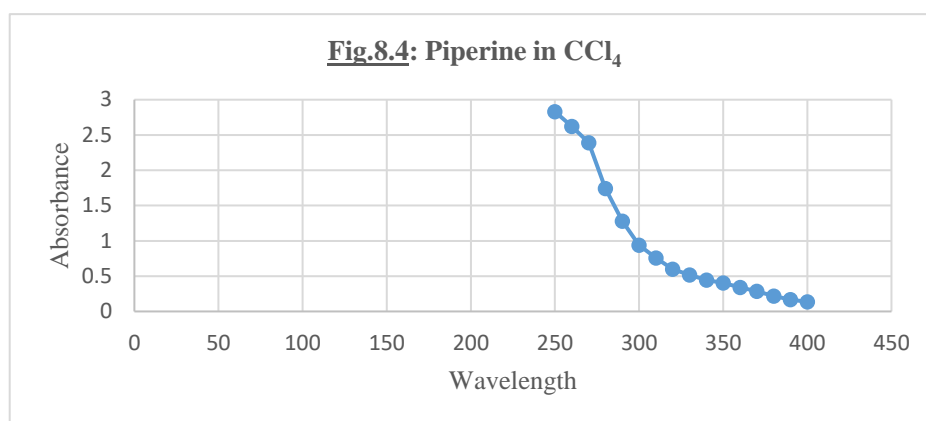


Fig.7: UV Visible Spectrophotometer type – 117

TABLE 1: Comparison of Piperine in UV with different solvents				
Wavelength	Ethanol	Methanol	Acetone	CCl₄
250	3	3	3	2.83
260	3	3	3	2.62
270	3	3	3	2.39
280	2.77	2.94	3	1.74

290	2.44	2.863	3	1.28
300	2.41	2.862	3	0.94
310	3	3	3	0.76
320	3	3	3	0.6
330	3	2.81	3	0.516
340	2.9	2.8	3	0.447
350	3	3	3	0.404
360	3	3	3	0.341
370	3	3	3	0.288
380	2.6	2.69	2.53	0.218
390	2.58	2.64	2.12	0.168
400	2.49	2.78	0.779	0.136





The UV Visible spectroscopy was performed after the IR spectroscopy, where the obtained Piperine sample was subjected to UV in different solvents. Solvents were chosen according to the solubility factor in Piperine. Ethanol, Methanol, Acetone and Carbon Tetra Chloride were taken as solvents for UV Vis analysis. At first Ethanol was taken with the crystallized piperine sample in a clean Quartz Cuvette and was subjected to UV Visible Spectrophotometer at different UV range. The data was recorded and the graphs were plotted accordingly. The similar procedure was followed to the other 3 solvents. In this Experiment Visible range was not seen as, the peak is obtained in UV range of each solvent.

In the project the UV spectral data is collected for the prepared piperine sample in different solvents like Ethnaol methanol acetone CCl₄. The peaks obtained requires in further interpretation and study. The project limits to providing a data of values alone in UV visible spectral region. For each solvent there is high scope of further study and more understandings. Each study can be done qualitatively to extract more information in order to give better interpretation of data.

According to the results obtained in the IR graphs, the peaks are high in the wavenumber 1250cm⁻¹ which is most common feature in piperine, IR studies and this is the region where it shows Carbon-Hydrogen stretching phenomenon.

In our study the graphs obtained are similar to the standard graphs of normal IR and also KBr discs.

The Infrared region of the electromagnetic radiations begins with 0.75 μm to 400 μm. The region of 2.5-16 μm in the IR spectroscopy provides the most vital chemical information. Instrumentation of the IR region is simple and effective. Deformation is caused as a result absorption of radiation by the molecules. Molecules either undergo stretching or bending. The Stretching occurs when excitation causes frequency of vibration changes in the bonded atoms along the bond axis. The sum of energy necessary to cause stretching or bending depends on the masses of atoms or group of atoms and on the hybridization of the atoms making up the bond. Several functional groups such as Carbonyls, hydroxyls, and double bond gives rise to characteristic absorptions in the group. The Frequency region of IR spectrum is 4000-1500 cm⁻¹. Bond vibrations in hydroxyl [OH] CH and NH bonds occur n 3600-2800 cm⁻¹ range. Carbonyl SH bonds, amino acids, triple bonds and cumulative double bonds absorb in 2850-1850 cm⁻¹ range. 1500-850 cm⁻¹ region is called fingerprint region. Spectra is more complicated in this region and identification of all the bands is impossible since numerous bond deformation and group vibration occur here. These bands are particular for a molecule being studied than the bands that appear in the group frequency region. Comparison of two IR spectra in this region provides positive structural identification. Common procedure does not exist for evaluating spectra. The absorptions of IR are very complicated. A complete vibrational analysis can be carried out for the sum of molecule using computers. Advanced computers are used for the analysis of molecules. The spectrum of interest can be sorted by comparison to spectral libraries. Tables for the characteristic bond and group frequencies can be used to help construct a structural formula. In this experimental study the spectrum of known compound is obtained then compared to a table of expected absorption bands for the molecules various groups and bonds.

Piperine can be extracted from black pepper and other piper species. The amount of piperine in black pepper is 9% by weight. Piperine is tasteless but its stereoisomer Chavicine is the active ingredient in black pepper that provides its characteristic taste. Loss of pungency during storage of black pepper is attributed to the slow isomerization of chavicine into piperine. [7]

IV.CONCLUSION

Even though solvent extraction methods are available to extraction of lycopene the method of ethanol extraction is employed frequently the UV Vis studies in different solvents present work shows various structural changes within the piperine molecule that requires further more thorough investigation however in context to IR spectra the stretching and bending within the molecule is in compliance with expected absorption bands The most prominent of spectral data is at wavelength 1580 which gives phenomenon of stretching of C-O-N bonds another prominent feature is at 1240 wavenumber indicating asymmetrical stretching of C-O-C bond the ATR-IR without KBr disc was carried out and there is further scope for investigation and interpretation of IR spectral data.

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