Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.122

CHARACTERIZATION OF PHYTOCHEMICALS ISOLATED FROM *CUCURBITA PEPO* SEEDS USING UV–VIS AND FTIR SPECTROSCOPY

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(Date of Receiving-02-01-2021; Date of Acceptance-18-04-2021)

ABSTRACT

C. pepo has a large range of application as herbal medicine and can be utilized as food and nutraceutical product due the presence of numerous bioactive phytochemical constituents. The present study was conducted to characterize various bioactive phytochemicals in Cucurbita pepo seeds using UV-Vis spectroscopy and phytochemicals isolated from *C. Pepo* using FTIR. The preliminary phytochemical screening showed that crude extract was rich inalkaloids, flavonoids, phenols, saponins, and terpenoids. The extract showed visible peaks at 293, 312, 299, 283 and 357 nm wavelength with the absorption 0.966, 1.012, 0.866, 0.954, 0.854 respectively. The FT-IR spectrum showed the presence of respective phytochemicals isolated from *C. pepo* seeds. This study showed the significance of UV-VIS and FTIR for identification of various phytochemicals in *C. pepo* seeds. Moreover, biologically active isolatescan be further analysed to investigate theirdiverse biological activities depending on their therapeutic applications in the pharmaceutical industries.

Keywords: FTIR, UV-VIS spectroscopy, Phytochemicals, *C. pepo* seeds

INTRODUCTION

Medicinal plants display a diverse range of biological properties and plant-based medicines gained a significant attention in the last few years, more so after COVID-19 pandemic era. Furthermore, it also provides a low cost, easily accessible, and safe method of treatment without side effectsas compared to synthetic drug (Sofowora *et al.,* 2013). The different phytoconstituents responsible for the therapeutic activity of medicinal plantsare flavonoid, alkaloid, phenol, tannins, carboxylic acids, terpenes, amino acids, andinorganic acids (Saxena *et al.,* 2013). Therefore, the analysis of these chemical constituents would help in determining innumerablebiological activities of plants,however, till now many plant species remain to be screened for their phytochemical composition and therapeutic benefits.

Pumpkin *(Cucurbita pepo*) is one of the well-known plants belonging to the family Cucurbitacae, widely grown for its nutritional value and vegetable purpose. Different parts of pumpkin such as flowers, fruits,seeds, and young leaves are edible and consumed by human since antiquity. In addition, pumpkin seedsare the nutrient rich foods and with high protein, low fat and a good source of minerals like Mg, Zn, Cu, Mo, and Se, *etc.*(Stovel, 2005; Yadav *et al.,* 2010). Pumpkin seeds are also commonly used in culinary practices and consumed as a snack all around the world (Montesano *et al.,* 2018). A variety of techniques can be used to screen and determine phytoconstituents such as alkaloids, phenols, flavonoids, saponins, terpenoids etc., Spectroscopic techniques are the most popular and useful tools used for phytochemical screening. The Fourier Transform Infrared Spectrophotometer (FT-IR) isone of the most powerful, non-destructive tools for identifying the types of chemical bonds/functional groups present in the phytochemicals. Different phytoconstituents present in plants like flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes, amino acids and inorganic acids give specific distinctiveness and properties to plants (Parekh *et al*., 2007). Therefore, the analysis of these chemical constituents would help in determining various biological activities of plants. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identify functional groups (Grube *et al.,* 2008).

Ultraviolet–visible (UV-Vis)spectroscopic analysis is rapid and cost effective test to decipher herbal plants and products for its characterization, identification, and authentication. The absorption, transmission, and emission of UV-Vis light wavelengths by plants and plant products are primary indication for purity and phytochemicals composition. UV–Vis spectrum is also used for new drug development and toxicity studies. The prediction by UV–Vis spectrum helps to develop the method of isolation and purification of phytochemicals

(Joshi, 2012).

C. pepo is very commonly used in the traditional system of medicine against several diseases that attracts researchers for further investigation. However, there is a limited study on the application of FTIR spectroscopy and UV-Vis profiling for the validation of pumpkin seeds. Therefore, the present research work was aimed to conduct the preliminary phytochemical analysis using UV-VIS and FTIR spectrum profile of *C. pepo* seeds.

MATERIALS AND METHODS

Plant Material and Preparation of Phytochemical Extract

The seeds of *C. pepo* were obtained from the local market (Orgrain India, India: FSSAI LIC No.12218009000371). The crude extract of *C. pepo* seed containing total phytochemical (TPC) were prepared for UV-VIS analysis. Extraction of phytochemical*viz*.total phytochemicals, total alkaloid (TAL) (Gonzales *et al*., 2014), total flavonoid (TFL) (Yassine *et al*., 2015), total phenol (TPH) (Weidner *et al*., 2012), total saponin(TSA) (Chaturvedi *et al*., 2012), and total terpenoid(TTE) (Mawa *et al.,* 2016) from defatted *C. pepo* seeds were carried out using authentic methods for FTIR analysis.

UV-VIS Spectrum analysis

The extract was centrifuged at 3000rpm for 10min and filtered through filter paper. The sample was diluted to 1:10 with the same solvent. The extract was scannedfrom 190 to 1100 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1800) and the characteristic peaks were detected and recorded (Dhivya *et al*., 2017).

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is a tool for identifying the types of functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different extract of purified fraction of *Cucurbita pepo* seed was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of potassium bromide (KBr) pellet, to prepare sample discs. The thin pressed pellet was loaded in FTIR spectroscope (Agilent) and infrared spectra and peak values were recorded between 4,000–600 cm^{-1}. For each sample, 200 scans were averaged with a spectral resolution of 4 cm^{-1}. It took three minutes for a recording process. Then for a given material, a final average spectrum was calculated (Kumar and Ramaswamy, 2014).

RESULT AND DISCUSSION

Several techniques like FTIR, Chromatography, UV-VIS are available to determine and estimate the presences of phytoconstituents/phytochemicals in herbal extracts. In the present study analysis of phytochemicals isolated from *C. pepo* seeds were analysed using FTIR and UV-VIS techniques.

UV-VIS spectroscopy is a simple, cost-effective and rapid test for detecting phytochemicals. This technique uses light in the visible or its adjacent ranges of wavelength. The colour of the chemicals involved directly affects the absorption in the visible range. In this technique, molecules undergo electronic transitions in an electromagnetic spectrum (Gunasekaran, 2003). This method is very appropriate as well as convenient in detecting phytochemicals in crude extracts. It does not only shortens the time but also serve as a preliminary analytic technique for phytochemical analysis in crude extract before purification steps.

The results presented in Figure 1, showthe varied section of the UV spectra obtained from the crude extract of *C. pepo* seed. The UV-VIS profile of *C. pepo* seed extract was studied over 190 to 1100 nm wavelength because of sharpness of the peaks and proper baseline. The profile showed the peaks at 293, 312, 299, 283, 357 nm with the absorption 0.966, 1.012, 0.866, 0.954, 0.854 respectively. According to the previous studies, these absorption bands are characteristic for phytochemicals like alkaloids, flavonoids, phenols, saponins and terpenoids (Kalaichelvi and Dhivya, 2017)

Our findings also confirm the aforementioned studies. An investigation conducted by Patrica *et al.,* (2013) explained that alkaloid fraction showed maximum absorption at 281 nm in UV spectroscopy which is in resemblance to our findings (Barros *et al.*, 2013). Joshi *et al.,* (2012) have reported that UV-VIS spectra of flavonoid and related glycosides exhibit two strong absorption peaks at 300–380 nm and 230–290 nm(Joshi, 2012). Some other studies also conveyed that the flavonoids and terpenoidsspectra typically consist of two absorption maxima in the ranges 230-285 nm (band I) and 300-350 nm (band II) which is in agreement with our results (Kalaichelvi *and* Dhivya, 2017; Renuka *et al.,* 2016). Malik *et al.,* (2018) revealedthat terpenoids were found at 372, 306, 248 nm with absorption 0.764, 0.912, 0.921 respectively (Malik *et al.,*2018) which

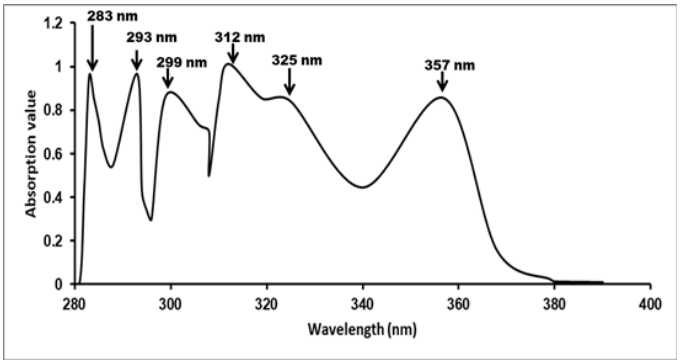
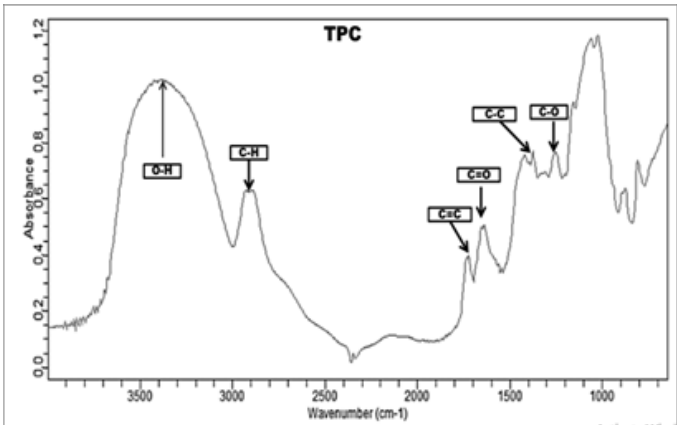
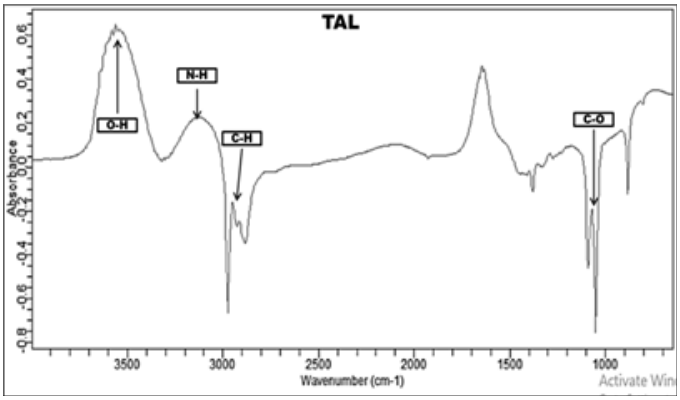
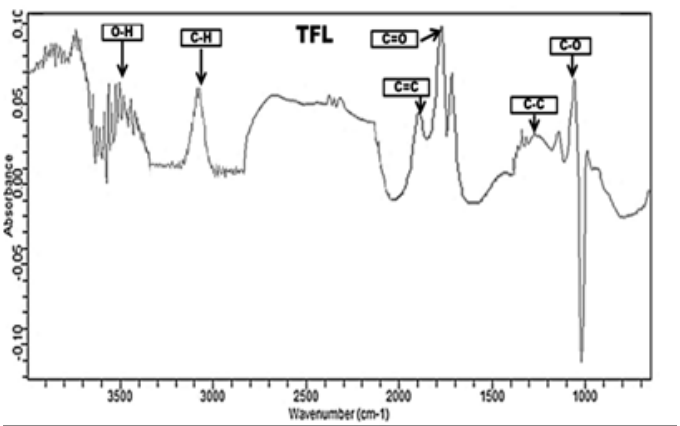
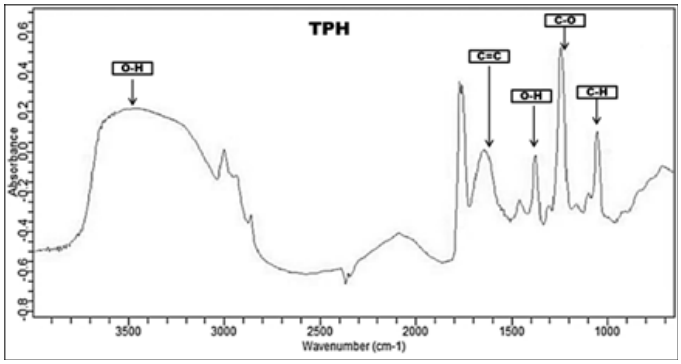


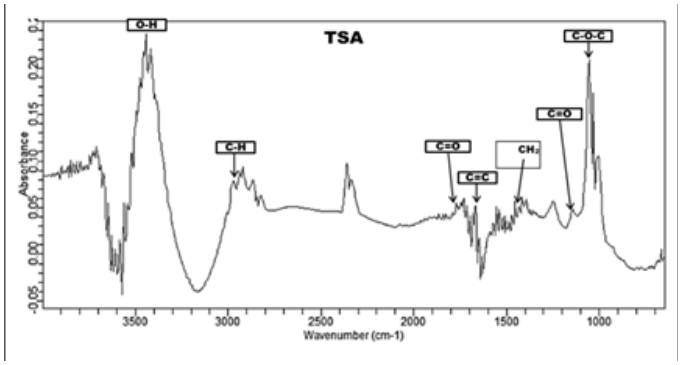
Figure 1: Preliminary analysis of crude extract of *C. pepo* seeds usingUV- VIS Spectrophotometry.











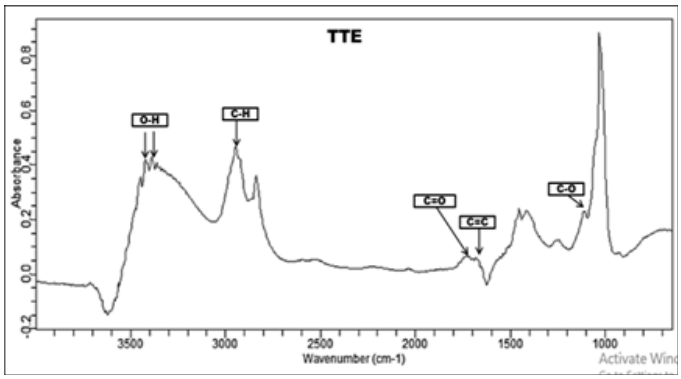


Figure 2: Fourier-Transform Infrared Spectroscopy analysis of total phytochemical (TPC), total alkaloid (TAL), total phenol (TPC), total saponin (TSA) and total terpenoid (TTE) of *C.pepo* seeds

also showing likeness with our results (Table 1). Our outcome of phenol spectra are in agreement of many other researchers (Engida*et al.,* 2015; Song *et al.,* 2015; Rojas, *et al*., 2016; Ebrahimia, *et al.,* 2015) who found that phenolic acids, e.g., gallic acid, ferulic acid, p-coumaric acids, and vanillic acid, have absorbance maxima in the range of approximately 290–350 nm. UV- VIS analysis of saponin extracted from sea cucumber showed that the maximum wavelength of saponin extract was at 282 nm and in case of standard saponin it was at 283 and 305 nm (Gonzalez-Valdez *et al.,* 2013; Barkya *et al*., 2016). Our findings are in accordance with the previous studies.

Fourier Transform Infrared (FTIR) Spectroscopy is a high-resolution analytical technique used to identify the chemical constituents of biomolecules (Kumar and Prashad, 2011). This spectroscopy is based on the measurement of the molecular bond vibration compounds, excited by radiation of a suitable frequency when given the conditions for energy absorption by the molecules (Singh *et al.,* 2011). FTIR offers a rapid and non-destructive investigation to fingerprint plant extracts or powders (Hashimoto and Kameoka, 2008).

In the present investigation,FTIR analyses were carried out by FTIR spectrometers (Agilent) and the scans range were between 4,000–600 cm^{-1}. The infrared spectrum was obtained to identify the characteristic absorption peaks corresponding to stretching vibrations of different functional groups. The appearance of FTIR bands in the range of 4000–1500 cm^{-1} represents a functional group and the presence of strong absorption bands in the region of 1500–600 cm^{-1}characterize the fingerprint region. Results of FTIR peak values and functional groups of *C. pepo* seedsare presented in Fig 2.

The FTIR spectroscopic analysis of the crude extract of *C. pepo* seeds showed the presence of various functional groups indicating the presence of bioactive compounds especially alkaloids, flavonoids, phenols, saponins and terpenoids. The IR spectra of crude extract containing total phytochemical (TPC)(Figure 2) of *C. pepo* seeds showed the presence of O-H stretching range from 3000-3700cm^{-1}, C-H stretching range from 2800-3000cm^{-1}, C=C peak from range 1700-1800, C=O peak from range 1600-1700cm^{-1},C-C peak from range 1400-1450cm^{-1}, C-O peak from range 1200-1300cm^{-1}. Previous studies that used FTIR technique to confirm the presence of various phytochemicals extracted from different parts of the plant are in agreement of our results as the peak and absorption were found in the same ranges that were found in our results (Lingegowda *et al*., 2012).

The IR spectra of TAL (Figure 2) showed the presence of alkaloids in the extractdue to presence of O-H stretching from range 3300-3600 cm^{-1}, N-H stretching from range 3000-3300, C-H peak from 2900-3000 cm^{-1} and C-O peak range from 1100-1200 cm^{-1}. Our finding of the total alkaloid (TAL) are in agreement of many researchers as their investigation showed that alkaloid compounds have major functional groups O-H, N-H and C-H stretching and bending groups (Son *et al.,* 2005; Windono *et al.,* 2012;Shami *et al.,* 2016). IR spectra of hordenine (alkaloid) showed O-H stretching vibration at 3733 cm^{-1}, N–H stretching vibrations are normally observed in the region 3200-3600 cm^{-1}. Some literature has reported that the C–H stretching vibrations are usually observed in 2800– 3200 cm^{-1}region. (Apoorva *et al.,* 2014).

The IR spectra of TFL (Figure 2) revealed the presence of flavonoids as O-H peak range from 3400-3500 cm^{-1}, C-H peak range from 300-3100 cm^{-1}, C=C peak range from 1850-1900 cm^{-1}, C=O peak range from1700-1850 cm^{-1}, C-C stretching range from 1150-1300 $cm^{-1}$and C-O peak range from 1000-1100 cm^{-1}.An IR spectra finding of our sample for flavonoid (TFL) is in corroboration with the studies reported previously. According to experimentation conducted by Noh *et al.,*revealed that flavonoids classes and their derivatives were found in the wavenumber range of 3500-3200 cm^{-1}, 3200-2800 cm^{-1}, 1800-1600 cm^{-1} and 1900-1500 cm^{-1} for flavonol, 1800-1600 cm^{-1} and 1900-1500 cm^{-1} for flavone and 1300-800 cm^{-1} for flavanone (Noh *et al.,* 2017). In other investigation, it was testified that IR spectroscopy highlighted the wavenumber range for the structure of the flavonoids at the different functional group that are C=O (1630 and 1665cm^{-1}), C-O (1000 and 1350cm^{-1}) and C-H (600 and 980cm^{-1}) (Trifunschi *et al.,* 2015).

In case of TPH(Figure 2), functional groups present were O-H stretches ranges from 3100-3700 cm^{-1}, C=C peak ranges from 1600-1650 cm^{-1}, O-H peak ranges from 1300-1400 cm^{-1}, C=O peak ranges from 1200-1300 $cm^{-1}$and C-H stretch with a range from 1000-1100 cm^{-1} respectivelySome investigation illustrates that a broad strong band was observed from a range 3270 ~ 3320 cm^{-1}due to the O–H stretching vibration of the phenolic group (Senthilkumar *et al.,* 2017; Orcic *et al.,* 2011). Our results of TPH is in agreement with the previous research that reported the spectral features of phenol is also characterized by the interaction of O–H deformation and C–O stretching vibrations in the spectral range between 1405 and 1220 cm^{-1}. It is also statedby several investigators that phenols are also represented with a doublet at 1640 cm^{-1} assigned to aromatic ring C=C stretching and aromatic C–H deformation vibration at 1110 cm^{-1}(Socrates, 2001; Taiwo *et al.,* 2018; LidijaSvecnjak *et al.,* 2009).

The IR spectra of TSA (Figure 2) showed -OH peak range from 3400-3500 cm^{-1}, C-H peak range from 2950-3000 cm^{1}, C=O peak at 1750 and 1100 cm^{-1}, C=C peak at 1650 cm^{-1}, CH_2 peak at 1450 $cm^{-1}$and C-O-C peakat 1000-1100 cm^{-1}. Our result is also in resemblance of other studies that reported the range and functional group of saponins which is also corresponding to our findings. One of the studies demonstrated the functional group present in crude extracts of soapnuts were OH, C=O, C-H, and C=C (Meshari *et al.,* 2014). FTIR spectrum of the purified soybean saponin indicated characteristic absorption peaks of saponin. It showed the presence of the long sharp peak at 3400 cm^{-1} indicates the presence of

hydroxyl groups (-OH), the peak at 2927 cm^{-1} represents alkyl groups (C-H), (–C=C) groups at 1628 cm^{-1}. C-O-C bond at 1052 cm^{-1}. The existence of -OH, C-H, and C=C bands in absorption peak of FTIR spectrum was characteristic of saponins. The C-O-C absorptions indicated glycoside linkages to the sapogenins(Mai *et al.,* 2019). In IR spectra of *Sapindusemarginatus*extract represent the functional group at different wavelength like (-OH stretching 3435), 2928 (-CH3), 1731(C=O stretching of acid), 1636 cm^{-1} (C=C Stretching), 1043 cm^{-1} (C-O starching of carbon-oxygen singles bond) (Bajad *et al*., 2016). The above infrared functional group absorptions characteristic of saponins were also mentioned in other studies also (Silva *et al.,* 2002; Kirmizigul *et al.,* 2002; Sengmin *et al.,* 2001; Lima *et al.,* 2002; Natori *et al.,*1981).

The presence of terpenoid revealed due to the presence of –OH range from 3000-3500 cm^{-1}, C-H from range 2900-3000 cm^{-1}, C=C from range 1600-1700 cm^{-1}, C=O from range 1700-1800 cm^{-1} and C-O from range 1100-1150 cm^{-1} in case of TTE extracted from the seed of *C. pepo.* It was already reported by Mohandas *et al.,*that terpenoids were present with C=O stretch in 1714.72 cm^{-1}in petroleum ether extract, 1726.29 cm^{-1}in ethyl acetate, chloroform and methanol extract and with O-H stretch in 3409.29, 3456.44 and 3431.36 cm^{-1}in petroleum ether extract and 3404.36, 3454.51, 3431.3 cm^{-1}in methanol extract (Mohandas *et al.,* 2018). The presence of hydroxyl group (~3400cm-1) or an oxo group (saturated 1750-1700 cm^{-1}) indicates the presence of terpenoids (Nita *et al.,* 2014). It was also reported that in the methanolic extract of *Rhapisexcelsa*C-H stretch at 2856.7- 2927.1 cm^{-1} also reveals terpenoids in terms of C=O stretch at 1704.18, 1708.04 cm^{-1} (Vanaja *et al.,* 2016). In IR spectra of linalool the presence of following functional groups were found C-C, C-O, C-H, and O-H stretching vibrations, peaks at 996, 1450, 2972 and 3569 cm^{-1} (Xiao *et al.,* 2017). These studies are in corroboration of our result.

CONCLUSION

In the present investigation, the analyses of the crude extract and phytochemicals extracted from *C. pepo* seeds were performed using UV-VIS spectroscopy and FTIR technique. The result indicates the presence of phytochemicals like alkaloids, flavonoids, phenols, saponins and terpenoids in *C. pepo* seeds. These phytochemicals may be one of the contributing factors responsible for properties like anticancer, antioxidant, anti-inflammatory, analgesic, and antimicrobial activities. Since these phytochemicals havevarious health benefits, the present study could be a promising investigation to identify the phytochemicals of *C. pepo* seeds and can be futherscreened for different kind of biological activities and disease mangenment depending on their therapeutic uses.

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