FT-IR Identification of Capsaicin from callus and seedling of chilli pepper plants Capsicum annuum L. in vitro.

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Abstract

Capsaicin spectrum was chemically characterized using vibrational spectroscopy identification from callus which initiated from different explants of local chilli pepper (Capsicum annuum L.) using Fourier Transform Infrared Spectroscopy (FT-IR). Analysis of Capsaicin alkaloid by Infrared Spectroscopy for callus derived from placenta and callus derived from shoots of 3 weeks old seedling pepper exhibits various characteristic of band which in turn confirmed the presence of a major peaks of amide, phenol, alkenes and ether functional groups.

Keywords: Chili pepper, In vitro, callus, capsaicin and FT-IR

1. Introduction

Plants are the basis of traditional medicine system for thousands of years[Bidumadhavi et al., 2009]. In fact many of drugs were naturally obtained substances for example, Latex from the opium poppy, Papaver somniferum, is a commercial source of the analgesics, morphine, and codeine [Hussain et al., 2012]. Chili peppers are of great importance in Native American medicine, and capsaicin is used in modern Western medicine as a stimulant and pain reliever. [Makari et al., 2009]. According to [Taira et al.; 2012] Capsicum fruit considered as an important constituent of traditional Chinese medicine. [Yatung et al.; 2014; Maurya et al., 2014 ] reported that India is one of the leading chilli producing countries of the world with around one million tons per year.

Chili pepper is an important source of capsaicin alkaloid which gives the fruits a punchy test as well as capsaicin have pharmaceutical properties due to its medicinal application e.g against high cholesterol levels [Kempaiah et al.,2005], food industry and production of defensive sprays [Tilahun et al.;2013]. Based on [Salih,2006] Oil Extracted from Capsicum annuum L has an antimicrobial activity . Moreover, the genus Capsicum provides antioxidant compounds, such as phenolics and carotenoids [Troconis-Torres et al.; 2012.; Materska and Peruka.2005.; Nascimento et al.;2014] as well as cosmetic industries, color and flavors[Kumar et al.2011].

A novel protocol has been described for large scale production of principle compounds under limited area by using in vitro technique which allows improving yield and quality for drugs along the year overcome season growth and the climate changes.

The aim of our study is to detect the presence of capsaicin alkaloid in different parts of the local chilli pepper explants using FT-IR analysis.

Materials and Methods

The present research was conducted at The Ministry of Science and Technology/Directorate of Agricultural Research, Genetic Engineering Department during the years 2015-2016. Callus which induced from shoots of 3 weeks old seedling pepper and placenta of local chilli pepper explants were used in this experiment.

Chilli pepper seeds were obtained from a local source. Seeds and the fruits of local chili pepper were surface sterilized with 70% Ethanol for one min, submerged in 4% sodium hypochlorite (NaOCl) for 20 min then washed with Sterile distilled water trice 5 min each. Seeds were germinated on [Murashige and Skoog,1962] inorganic salts medium , supplemented with 2 mg/L GA3, 3000 mg/L Sucrose. Three weeks later, shoots were cultured on Callus induction media which previously described by [El Kaaby et al.2015],wear as the placenta were separated from chilli sterilized fruits and cultured on the same previous medium with addition of 2 mg/L 2,4-D. Both callus and germination media were solidified with 6000 mg/L Agar. The pH of both media was adjusted to 5.75 before autoclaving at 120 C° for 20 min.
FT-IR studies

Sample preparation

In order to obtain a solid pellets sampling, a dried powder of the different chilli pepper explants were used for FT-IR analysis. Method previously described [Ashokkumar and Ramaswamy, 2014] with slight modification. Briefly, (1.5) mg of potassium bromide (KBr) were added to (1.5) mg of each drying samples afterwards, the mixtures of selected pure capsaicin standard and samples were loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a Scan range from (400 to 4000 cm$^{-1}$) with a resolution of (4 cm$^{-1}$).

Results and Discussion

FT-IR spectra and Characteristic peaks of (standard) capsaicin, callus derived from shoots 3 weeks old and callus derived from placenta are presented in fig (1,2 and 3) respectively. According to FT-IR spectral data in fig 1,2 and 3 revealed that all spectra show absorption bands in the range of (3315 – 3354 cm$^{-1}$) due to the presences of characteristic stretching vibrations of (N-H) in amino acids. Moreover, both samples and the standard capsaicin showed an aliphatic (C-H) stretching vibration in the range of (2926- 2866 cm$^{-1}$) also presences of (C=O) stretching vibration at the range (1633, 1639 and 1624 cm$^{-1}$) for the standard capsaicin and the two samples. All samples showed a range of (1442-1556 cm$^{-1}$) due to the presence of (C-C) stretching vibration in aromatic ring and bending out-of-plane (C-H) at range of (804-775 cm$^{-1}$). Also two vibrations were observed in both standard capsaicin and sample 3(fig 1, 3) due to the presence of asym stretching vibration in (C-O-C) at range of (1278-1247 cm$^{-1}$).

Conclusion

Capsaicin was successfully identified by using Fourier Transform Infrared Spectroscopy (FT-IR). However, recent research is concentrating on a fast detection of the natural active compounds in plants. Fourier transform-infrared spectroscopy (FTIR), is an essential technique which has been widely employed for medicinal plants [Ashokkumar and Ramaswamy, 2014; Maobe and Nyarango, 2013] human disease [Agrov et al., 2002] also forensic science [Kazarian et al., 2011] and food characterization due to its nondestructive nature as well as allows for easy identification [Troconis-Torres et al., 2012]. Our study was encouraging for detecting other relative compounds in other plants in short time and less cost using FT-IR detection.
References


