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# 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]. Chilli 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 chili 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 (standerd) capsaicin, callus derived from shoots 3 weeks old and callus derived from(placenta) are presented in fig (1,2 and 3 ) respectivily .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.

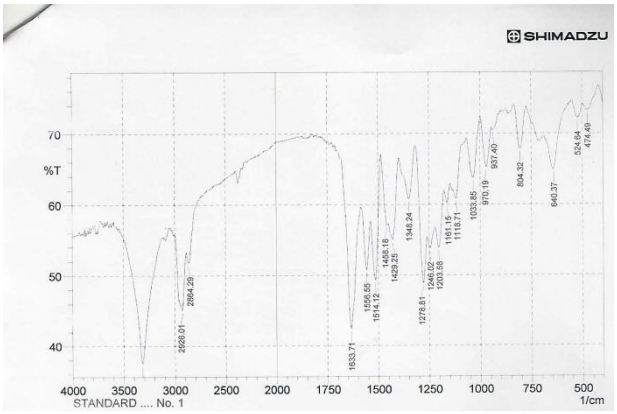


**Fig.1** Characteristic peaks (standerd)capsaicin: N-H stretch( 3315 cm$^{-1}$ ), aliphatic C- H stretch. (2926 cm$^{-1}$, 2864 cm$^{-1}$), C=O stretch.(1633 cm$^{-1}$),aromatic C-C stretch. (1556 cm$^{-1}$) and out-of-plane C-H bending (804 cm$^{-1}$), N-H bending and C-N stretch. (Amide II) (1514 cm$^{-1}$),asym C-O-C stretch (1278 cm$^{-1}$), C-O stretch (1203 cm$^{-1}$)

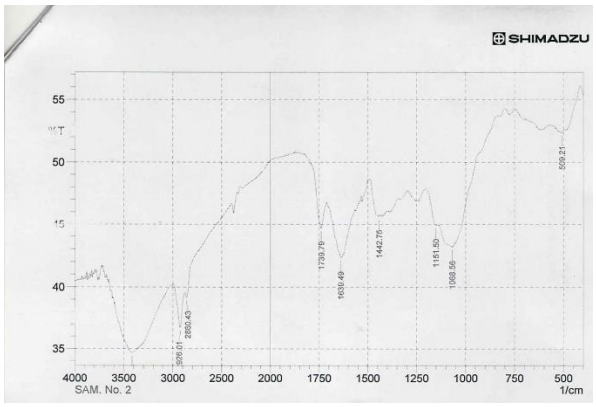


**Fig.2** Characteristic peaks of capsaicin in callus derived from shoots 3 weeks old : N-H stretch(3415 cm$^{-1}$ ), aliphatic C-H stretch. (2926 cm$^{-1}$, 2860 cm$^{-1}$), C=O stretch.(1639 cm$^{-1}$), aromatic C-C stretch. (1442 cm$^{-1}$)

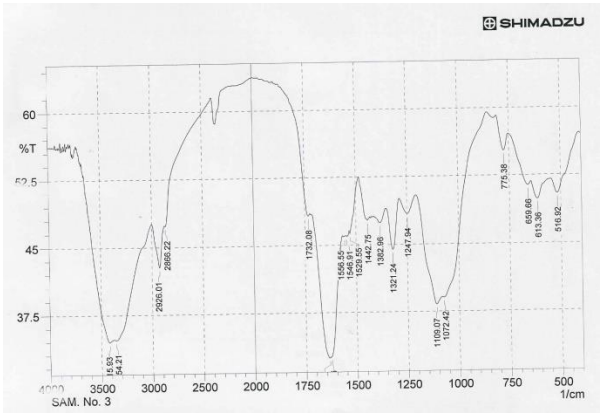


**Fig.3** Characteristic peaks of capsaicin in callus derived from(placenta): N-H stretch (3354 cm$^{-1}$ ), aliphatic C-H stretch.(2926 cm$^{-1}$, 2866 cm$^{-1}$), C=O stretch.(1624 cm$^{1}$),aromatic C-C stretch. (1556 cm$^{-1}$) and out-of-plane C-H bending (775 cm$^{-1}$), N-H bending and C-N stretch. (Amide II) (1529 cm$^{-1}$),asym C-O-C stretch (1247 cm$^{-1}$)

## References


1. Agrov.S.; Salman.J.R.A.; Goldstein.I.S.J.; Guterman.H and Mordechai.S.2002. Diagnostic potential of Fourier-transform infrared microspectroscopy and advanced computational methods in colon cancer patients. Journal of Biomedical Optics 7(2): 1–7.
2. Ashokkumar.R and Ramaswamy.M.2014. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. Int.J.Curr.Microbiol.App.Sci. 3(1): 395-406.
3. Bidumadhavi.B.; Ravindernath.A.; Banji.D.; Madhu.M.N.; Ramalingam.R and Swetha.D.2009. Extraction, Identification, Formulation and Evaluation of Piperine in Alginate Beads. Inter.J. of Pharmacy and Pharmaceutical Science.Vol.1(2):156-161.
4. El Kaaby, Ekhlas Abdulkareem jasim, Al-Ajeel, Saadon. Abdulhadi. and Al Hattab, Zahra Noori.2015. Effect of Plant Hormones on Callus Induction from Fruit and Seedling Explants of Chilli Pepper (*Capsicum annuum* L.). J.Life Sci. s9: 18-26.
5. Hussain.Md.S.; Fareed.S.; Ansari.S.; Rahman. Md.A.; Ahmad.I.Z. and Saeed.M.2012. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 4(1): 10–20.
6. Kazarian .S.G.; Ricci .C.; Boyd .S and Kansiz .M.2011. ATR FTIR imaging in forensic science. www.agilent.com/chem.
7. Kempaiah, R.K.; Manjunatha, H.; Srinivasan, K. 2005. Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats. *Mol. Cell. Biochem*, *275*: 7-13.
8. Kumar.R.; Dwivedi.N.; Singh. R. K.; Kumar.S.; Rai .V.P and Singh. M. 2011. A Review on molecular characterization of pepper for capsaicin and oleoresin. Inter.J. Plant Bre. & Gen.PP.5(2): 99-110.
9. Makari **H**. K.; H. S. R .Patil.; Abhilash. M. and Kumar H. D. M. 2009. Genetic diversity in commercial varieties of chilli as revealed by RAPD method. Indian. J. Sci. & Techno.2 (4):91-94.
10. Maobe.M.A.G and Nyarango.R.M. 2013. Fourier Transformer Infra-Red Spectrophotometer Analysis of *Warburgia ugandensis* Medicinal Herb Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. Global Journal of Pharmacology 7 (1): 61-68.
11. Maurya.V.K.; Vijaypratap M.; Ramesh.N.; Srinivasan. R and Gothandam.K.M.2014. Impact of salt on capsicin synthesis in three capsicum cultivars. R.J.P.B.C.S.5 (6) : 735-**7**40.
12. Materska.M and Peruka.I.;2005. Antioxidant Activity of the Main Phenolic Compounds Isolated from Hot Pepper Fruit (Capsicum annuum L.). J. Agric. Food Chem. Vol.53: 1750-1756.
13. Murashige, T., and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures." *Physiol. Pl.* 15: 473-497.
14. Nascimento .*P.L.A.;* Nascimento.T.C.E.S. Ramos.N.S.M.; Silva.G.R.; Gomes.J.E.G.; Falcão.R.E.A. Moreira.K.A.; Porto.A.L.F and Silva. T. M. S.2014. Quantification, Antioxidant and Antimicrobial Activity of Phenolics Isolated from Different Extracts of *Capsicum frutescens* (Pimenta Malagueta). *Molecules* . *19*:5434-5447.
15. Salih.A.A.2006. Extraction and Identification of Oil Extract from *Capsicum annuum* L. Fruits and Study of its Antimicrobial activity. J.Basrah Researches (Sciences) Vol.32. (3): 80 -87.
16. Taira.S.; Shimma.S.; Osaka.I.; Kaneko.D.; Ichiyanagi.Y.; Ikeda.R.; Konishi-Kawamura.Y.;Zhu.S.;Tsuneyama.K and Komatsu.K.2012. Mass Spectrometry Imaging of the Capsaicin Localization in the *Capsicum* Fruits.Inter.J. Biotech for Wellness Industries.1:61-65.
17. Tilahun .S.; Paramaguru .Pand Rajamani.K. 2013. Capsaicin and ascorbic acid variability in chilli and paprika cultivars as revealed by HPLC analysis. J. Plant Breed. Genet. 01 (02): 85-89**.**
18. Troconis-Torres**.;** Rojas-López.M.; Hernández-Rodríguez.C.; Villa-Tanaca .L.; Maldonado-Mendoza.I.E.; Lidia.DA. Tellez-Medina.M and Jaramillo-Flores.M.2012. Biochemical and Molecular Analysis of Some Commercial Samples of Chilli Peppers from Mexico J.Biomed & Biotech. (2012):1-11.
19. Yatung.T.; Dubey.R.Kr.; Singh.V.; Upadhyay.G. and Singh.S.2014. Studies on seed protein profiling in chilli (*Capsicum annuum* L) genotypes of Northeast India. Aus.J.C.Sci. 8(3):369-377.