MODIFIED METHODS OF EXTRACTION OF POLYENE ANTIOXIDANTS PIPERINE, CURCUMIN AND B-CAROTENE

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Submitted On: 26.03.2016	Revised On: 30.03.2016	Accepted On: 31.03.2016

ABSTRACT:

Antioxidants have shown marked effects on certain Mental diseases. Antioxidants bearing conjugated system like polyene class of antioxidants known to be powerful scavenger of free radical bear the ability to suppress the oxidative stress in the brain. Three polyene antioxidants viz Piperine, Curcumin and β-Carotene were extracted and isolated to their purest form, from the plants viz *Piper Nigrum, Curcuma Longa* and *Daucus Carota* respectively. The extraction of the phytoconstituents was based on modified reported methods. Standardization and characterization of extracted phytoconstituents was done using FTIR, NMR, MASS, UV spectroscopy and HPLC.

KEY WORDS: Polyene antioxidants, Piperine, Curcumin, β-Carotene, extraction.

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Indian Research Journal of Pharmacy and Science; 8(2016) 471-485
Journal home page: https://www.irjps.in

1. INTRODUCTION

In current situation antioxidant hold's vital importance in treating oxidative stress which is a marked cause for variety of neurodegenerative diseases like Alzheimer's, Depression, Epilepsy, Parkinson, Huntington, Schizophrenia, Anxiety etc.[1] The concentrations of Antioxidants in plants are low hence bulk plant products are used as a dose for the treatment of diseases. To have antioxidants in purest form to be used in low, they should be extracted and purified. Piperine, Curcumin and β-Carotene are Polyene antioxidants found in the plants viz *Piper nigrum, Curcuma longa, Daucus carota*. They have shown their antioxidant effects in lipid phases by free radical scavenging or singlet oxygen quenching.[2,3] In biological systems, polyene antioxidants convey their mechanism for both of singlet and triplet oxygen (chain-breaking antioxidants).[4] Polyene antioxidants are proven to be effective biological quenchers of Singlet oxygen which are responsible damaging DNA, lipids, proteins, and neurolipids.[5]

2. MATERIAL AND METHODS

Crude parts (collected from the Delhi local market and authentication voucher number ZC/2009/V21, T142, L13) of the selected plants were sorted out by hand to remove impurities and later were dried in air and in oven at 45°C temperature. Then the materials were coarsely powdered for extraction and isolation. Further their identification was done via HPLC (SHIMADZU, Japan), NMR (BRUKER, DRX-400 MHz, USA), FTIR (BIO-RAD FTS-6000, USA), MASS (JEOL SX 102\ DA-6000 (10 kV).

2.1 Extraction and characterization of Piperine from Piper nigrum

Coarse powder of black pepper (2 Kg) was extracted with 5 L 95% ethanol in a soxhlet extraction apparatus. It was cooled and filtered using Whattman grade 1 filter paper. Filtrate was Concentrated to 500 ml in a vacuum on a 50°C water bath to remove most of the ethanol solvent. 1.5 L of 10% alcoholic KOH was added and heated. Residue was left overnight. There after solution was decanted to form the insoluble residue. Water was added drop wise to form yellow crystals and left undisturbed overnight use, further long yellow needles of Piperine crystals deposited were collected by filtration.[6, 7]

2.2 Extraction and characterization of Curcumin from curcuma longa

Curcuminoids were isolated from 2 kg rhizomes. Briefly, turmeric powder was successively extracted with 95% ethanol at room temperature. Then curcuminoids (1 g) was mixed with silica gel (0.5 g) and separated using silica gel column chromatography (eluent: chloroform: (methanol: acetic acid), 98: 2 (8:2). Curcumin was eluted first and then eluent was dried as yellow powder and recrystallized with ethanol to give the yellow crystals of Curcumin.[8]

2.3 Extraction and characterization of β-carotene from Daucus carota

Carotenoids were extracted from 3 Kg fresh roots of *Daucus carota*[9] with acetone and petroleum ether mixture (1:1) at control temperature 40-60˚C using Soxhlet apparatus until the tissues were completely recolored. After removing the acetone and water content, the ether extract was dried with Na_2SO_4, and subjected to separation in separatory funnel and was concentrated under reduced pressure at 35˚C. Then β-carotene was isolated from the remaining carotenoids by the column chromatography method. Chromatography column (20 cm × 1 cm) was filled with $Ca(OH)_2$. To elute β-carotene, gradient solution of petroleum ether and acetone was used (0 to 2% of acetone) and was crystallized by concentrating and keeping it at low temperature.

2.4 Storage conditions

All the extracted phytoconstituents were kept in dark amber colored vial at -4±2 ºC.

3. RESULT

3.1 Characterization and Standardization of Piperine

Yield: 8.6 grams (0.43%), Melting Point: 130 - 131°C, UV absorption: λ_{max} peaks of standard and extracted sample matched at 343 nm, FTIR: Extracted peaks matches with the standard, HPLC: standard and extracted have same,

retention time: 4.2 minutes, mobile phase: methanol: water, Filter membrane: 0.2µ, flow rate: 1ml/min, wavelength: 343 nm, ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm, 1.58 (6H, d, 3×CH_2-piperidine -18, 19, 20), 3.58 (4H, s, 2×CH_2-piperidine-17, 21), 5.96 (2H, s, CH_2-dioxolane-2), 6.42 (1H, d, J=14.4 Hz- transene-13) 6.74 (2H, d, J=8.4 Hz- Aromatic-6,8), 6.78 (1H, d, J=12.8 Hz- 11), 6.87 (1H, d, J=7.2 Hz- ene-10), 6.97 (1H, d, J=1.2 Hz- Aromatic-9), 6.42 (1H, dd, J=14.4 Hz- transene-12). Mass spectra *m/z*: M^+ 285.2 (96%), 204.2 (100%), 172 (47.3%), 120.1(86.2%). (Figures 1-5)

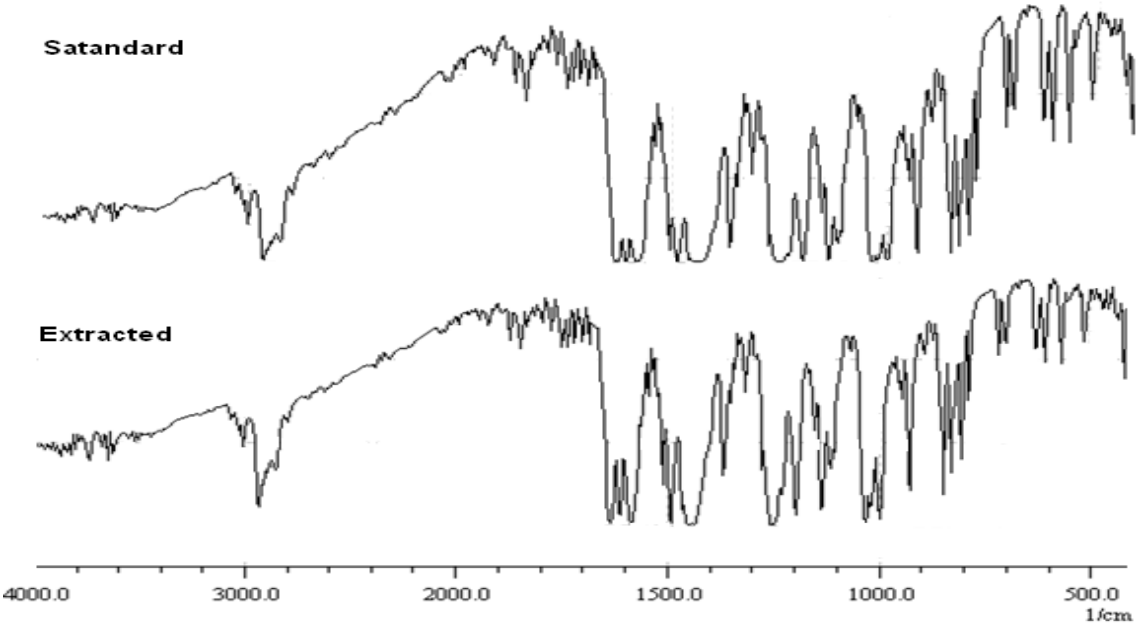

Figure 1. IR Standardization of Piperine

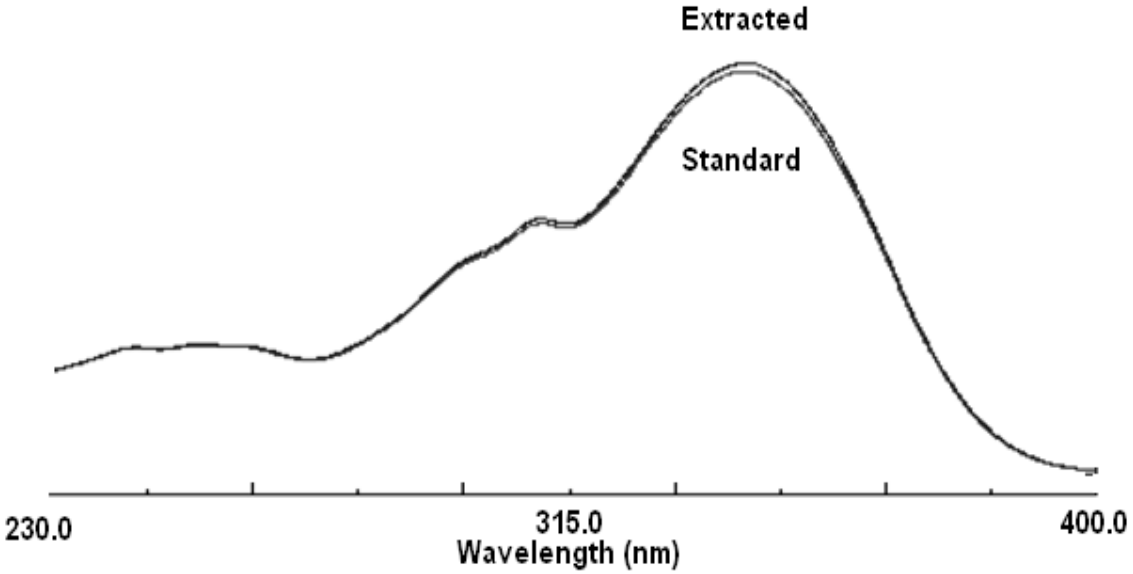

Figure 2. UV Standardization of Piperine

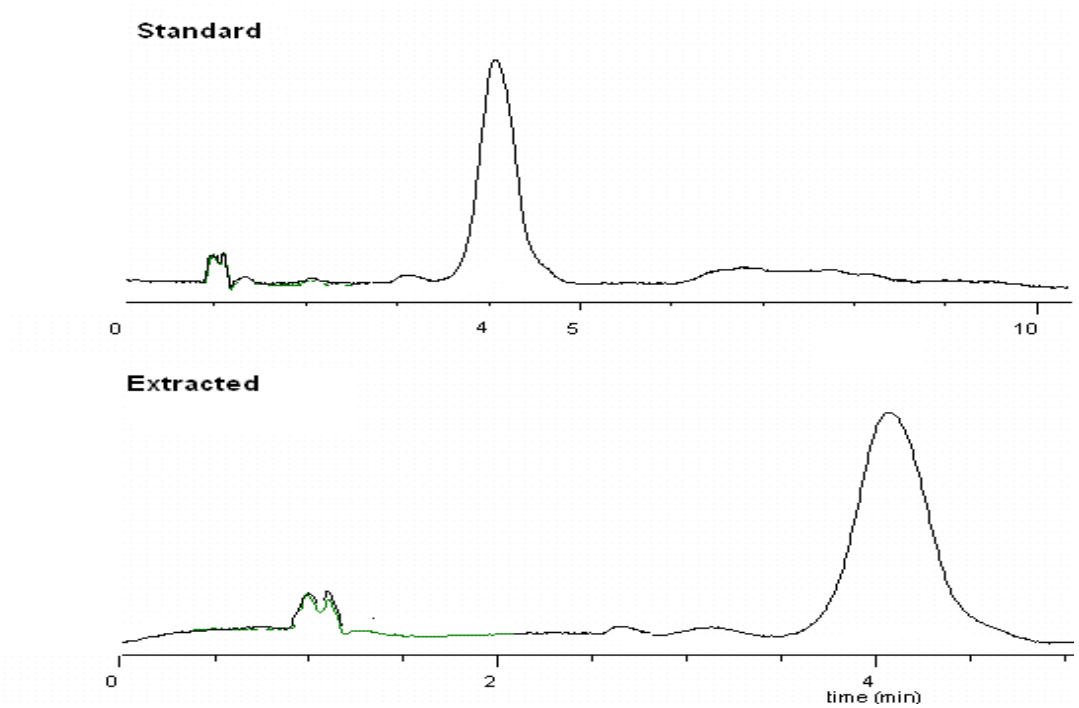

Figure 3. HPLC Standardization of Piperine

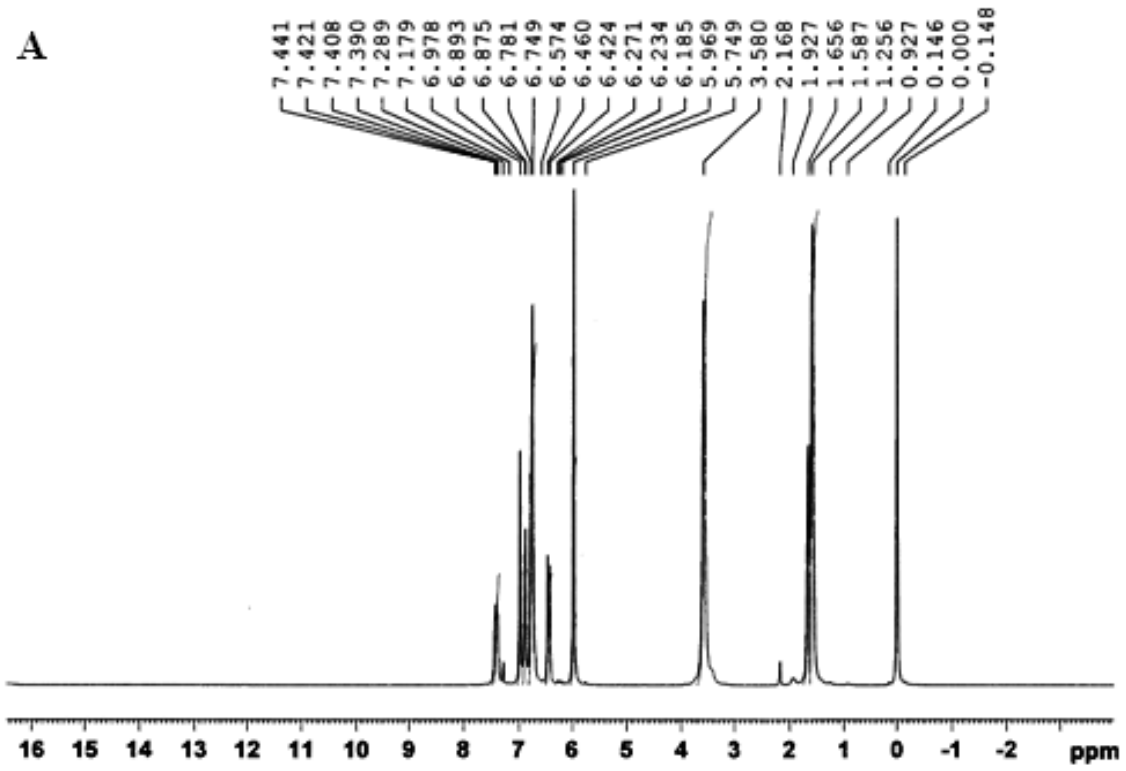

Figure 4A. NMR expanded form Characterization of Piperine

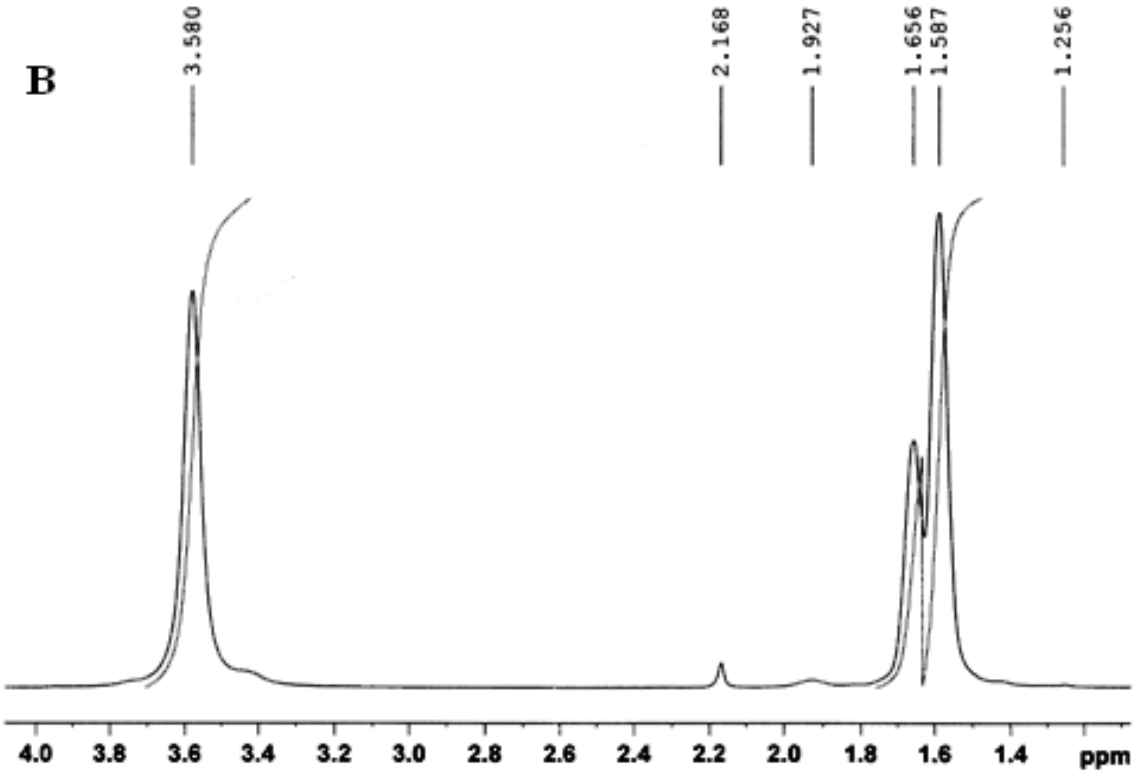

Figure 4B. NMR expanded form Characterization of Piperine

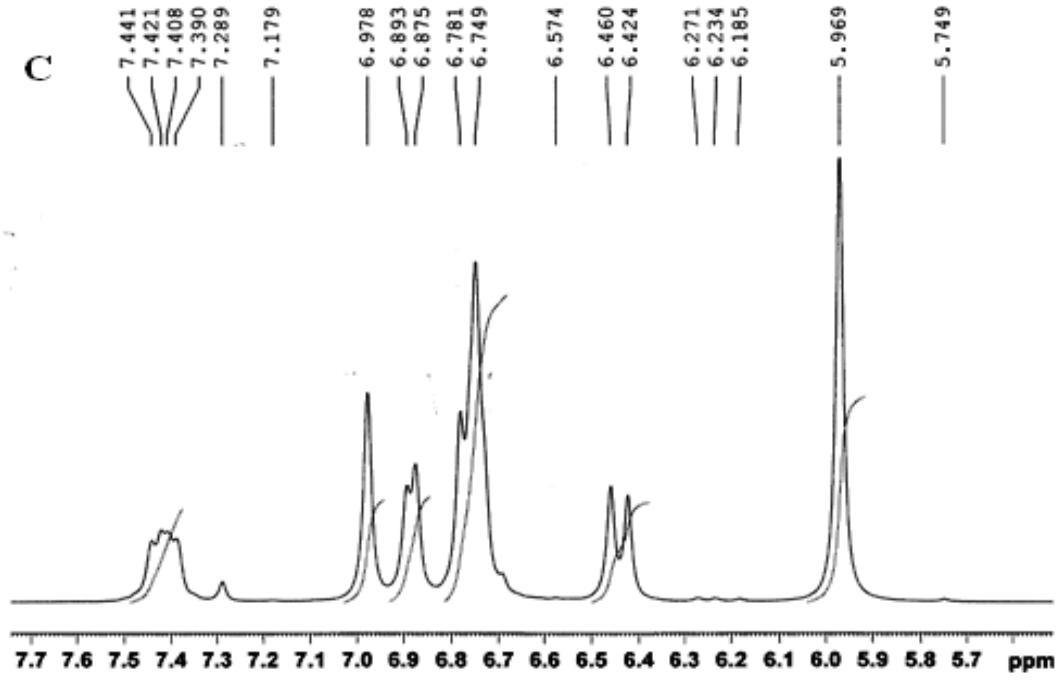

Figure 4C. NMR expanded form Characterization of Piperine

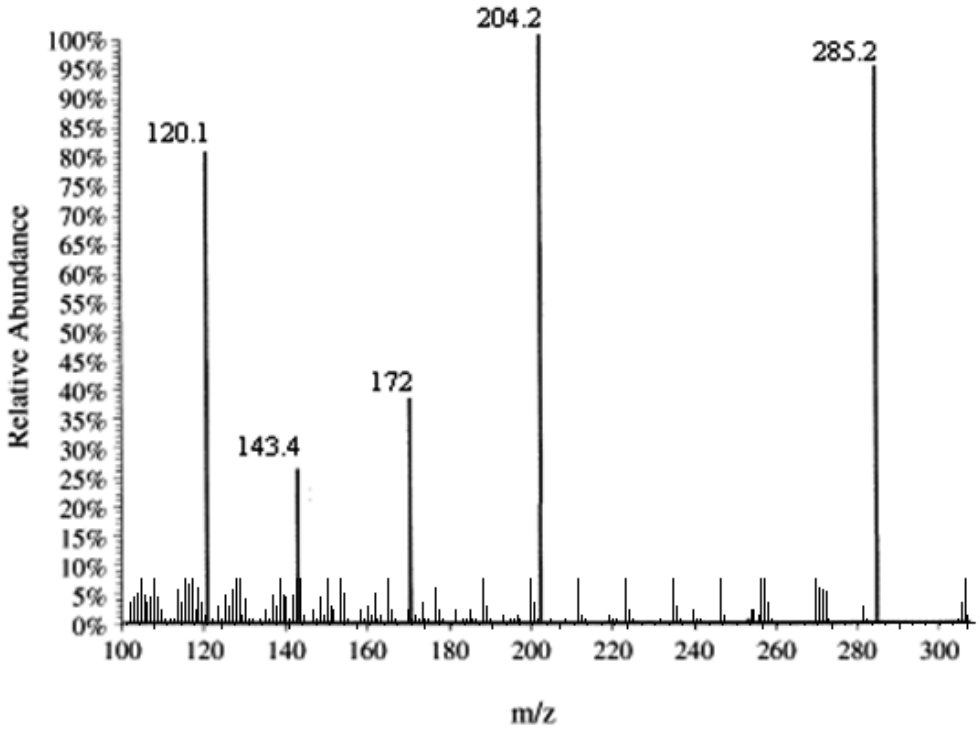

Figure 5. Mass Characterization of Piperine

3.2 Characterizationand Standardization of Curcumin

Yield: 0.60 g (0.03%), Melting Point: 185-187 °C, UV absorption: λ_{max} peaks of standard and extracted sample matched at 423 nm, FTIR: Extracted peaks matches with the standard, HPLC: standard and extracted have same retention time: 7.2, mobile phase Solvent A: Aqueous sodium acetate buffer (0.04 M); adjusted to pH of 3 using dilute orthophosphoric acid and Solvent B: Acetonitrile (v/v). Filter membrane: 0.45 µ, flow rate: 1.0 mL/min; wavelength; 425 nm, ^{1}H-NMR (400 MHz, d_6-DMSO, TMS) δ ppm 3.31 (2H, t, CH_2 -14), 3.91(6H, s, 2×OCH_3-8, 26), 4.87 (2H, s, 2×OH-9,27), 6.58 (2H, d, J=14.4 Hz- CH=transene-18,10), 6.81 (2H, d, J=10.0 Hz-Ar-4,24), 7.10 (2H, d, J=4.8 Hz-Ar-3,23), 7.21 (2H, s, Ar-6,20), 7.55 (2H, d, J=14.4 Hz-CH=transene-11, 17). Mass spectra**:** *m/z*: 369.1(M+1) (38.2%), 151.1 (100%), 177.4 (96.7%), 253.3 (47.2%), 284.8 (39.7%). (Figures 6-10)

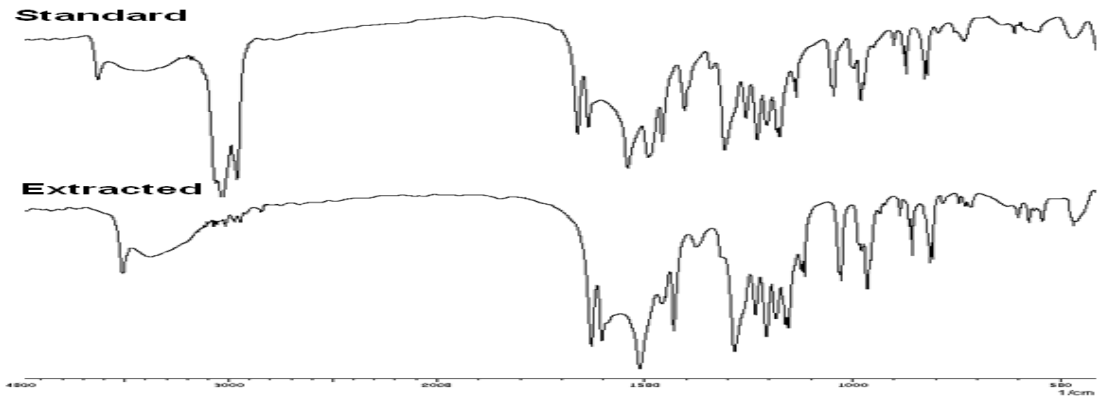

Figure 6. IR Standardization of Curcumin

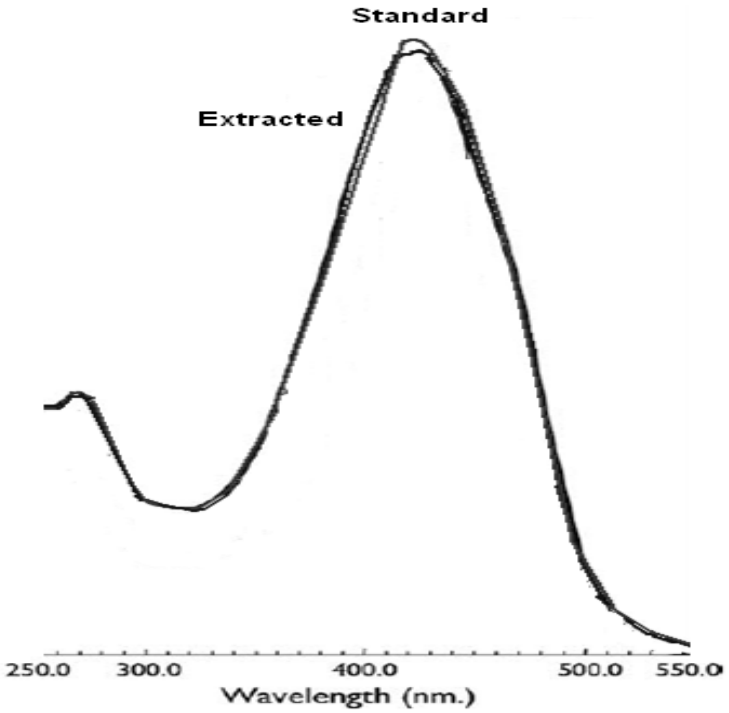

Figure 7. UV Standardization of Curcumin

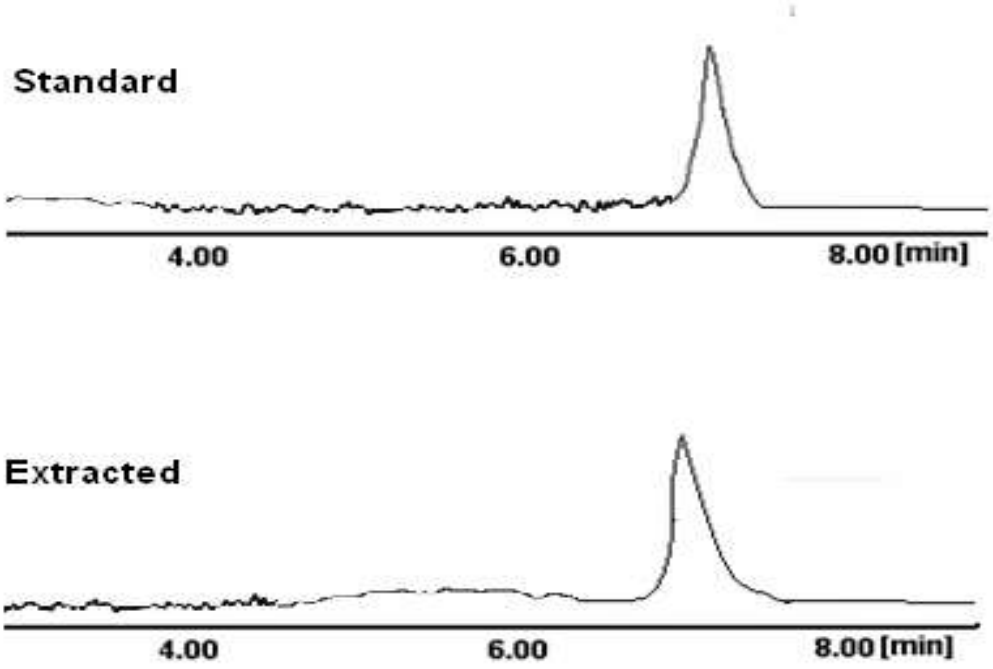

Figure 8. HPLC Standardization of Curcumin

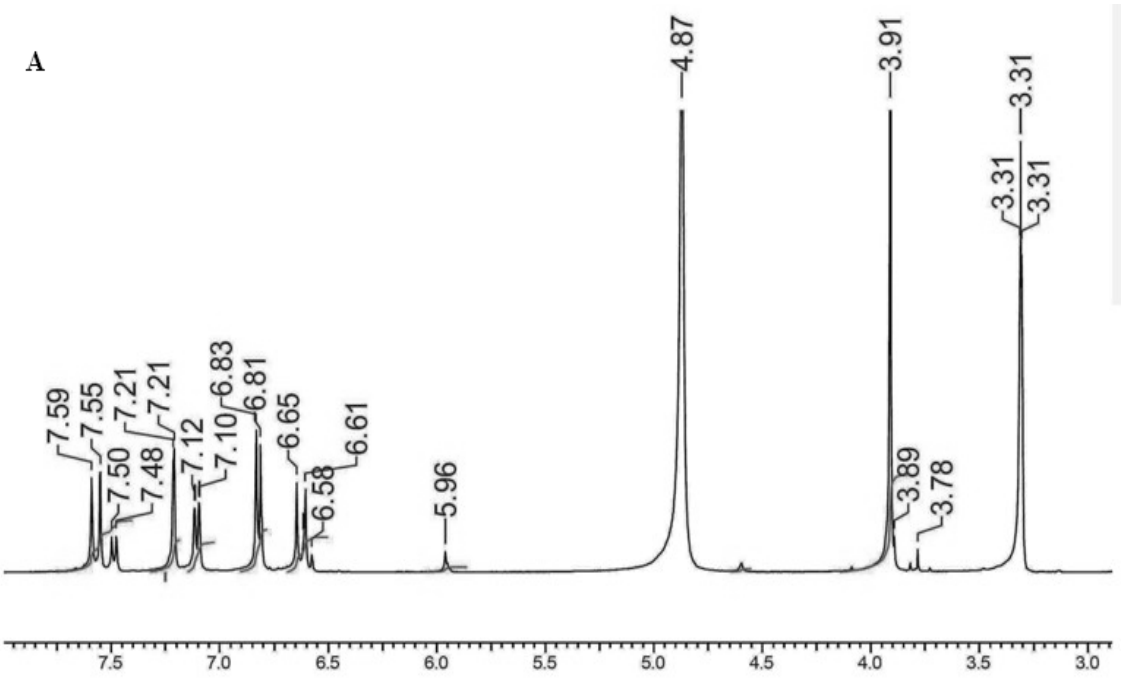

Figure 9.A. NMR Characterization of Curcumin

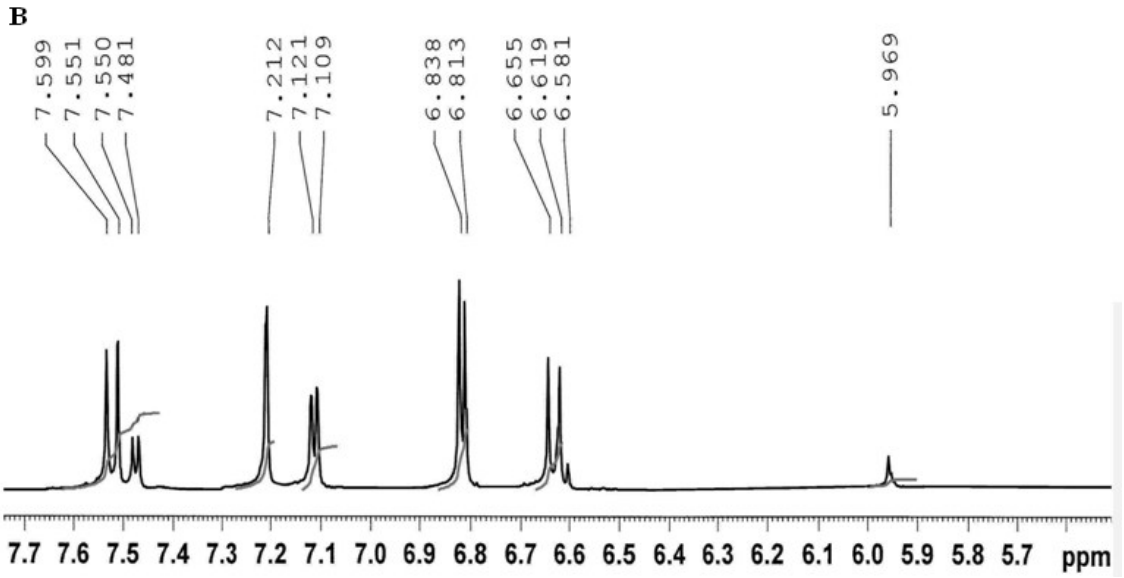

Figure 9 B. NMR Characterization of Curcumin

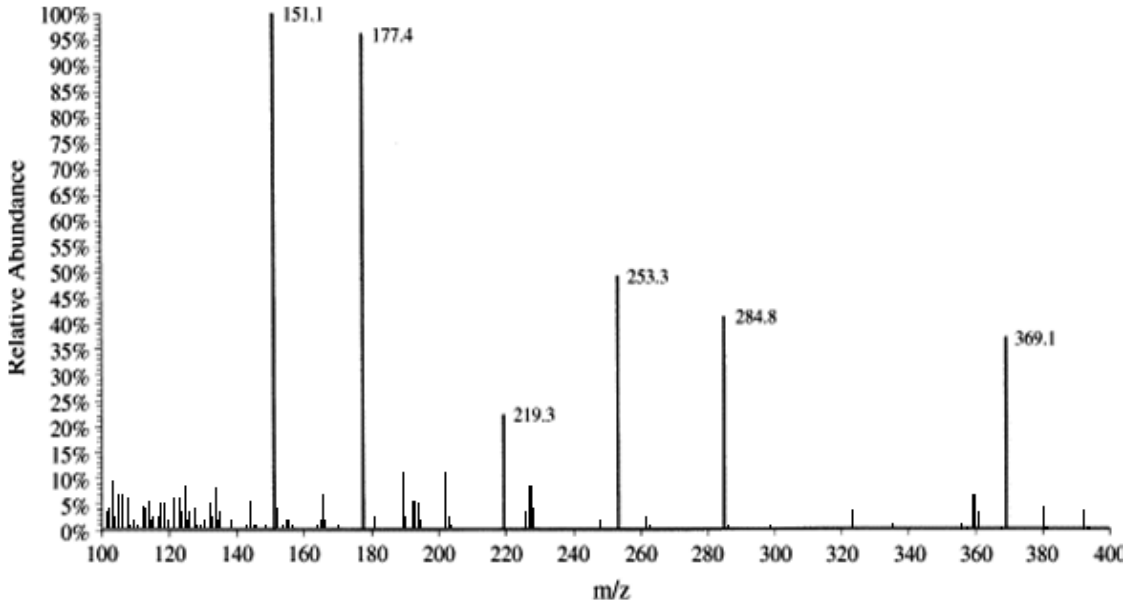

Figure 10. Mass Characterization of Curcumin

3.3 Characterization and Standardization of β-carotene

Yield: 1.256 g (0.041%) Melting Point: 181˚C, UV absorption: λ_{max} peaks of standard and extracted sample matched at 450 nm, FTIR: Extracted peaks matches with the standard, HPLC: standard and extracted have same retention time: 4.7, mobile phase: Acetonitrile, dichloromethane and methanol (70:20:10), Filter membrane: 0.45 µ, Flow rate: 2 ml/minute, wavelength: 450 nm. ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm 1.45 (4H,ddd, J=4,4,4-Hz-2×CH_2-25,34), 1.59 (4H,qd, 2×CH_2-26,35), 1.72 (12H, s, 4×CH_3-29,31,38,40), 1.97 (18H, s, 6×CH_3-5,10,16,21,28,36), 2.01 (4H,dd, J=4,4-Hz-2×CH_2-24,33), 6.11-6.65 (14H, 2×m, J= 4, 4, 8, 12, 4, 4, 4, 7×CH=CH conjugated double bonds protons) Mass spectra *m/z*: 537 (100.0%), 538 (43.9%), 539 (9.1%). On the basis of all spectral and HPLC data, present compound was characterized to β-Carotene. (Figures 11-15)

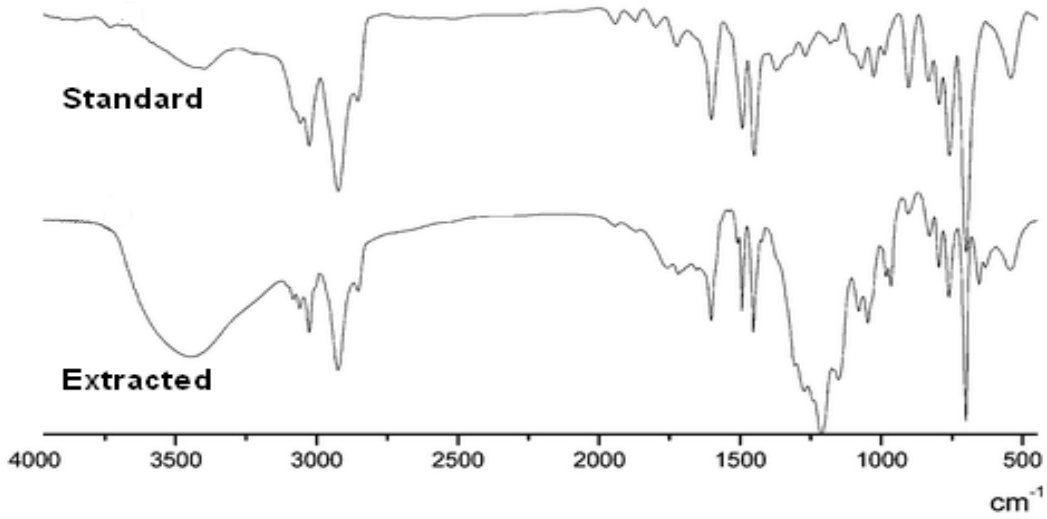

Figure 11. IR Standardization of β-carotene

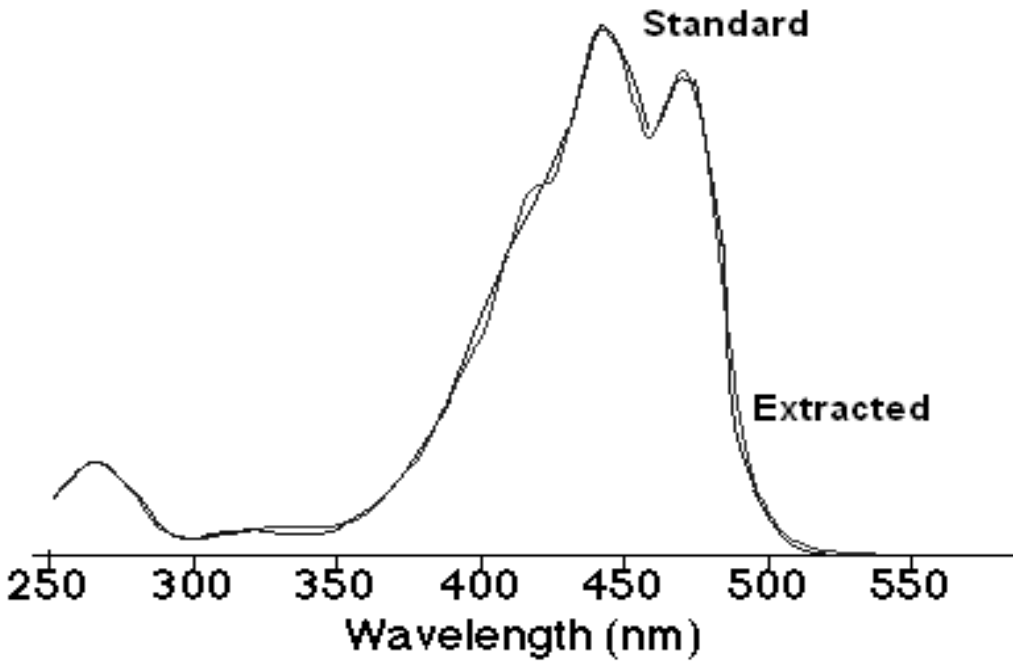

Figure 12. UV Standardization of β-carotene

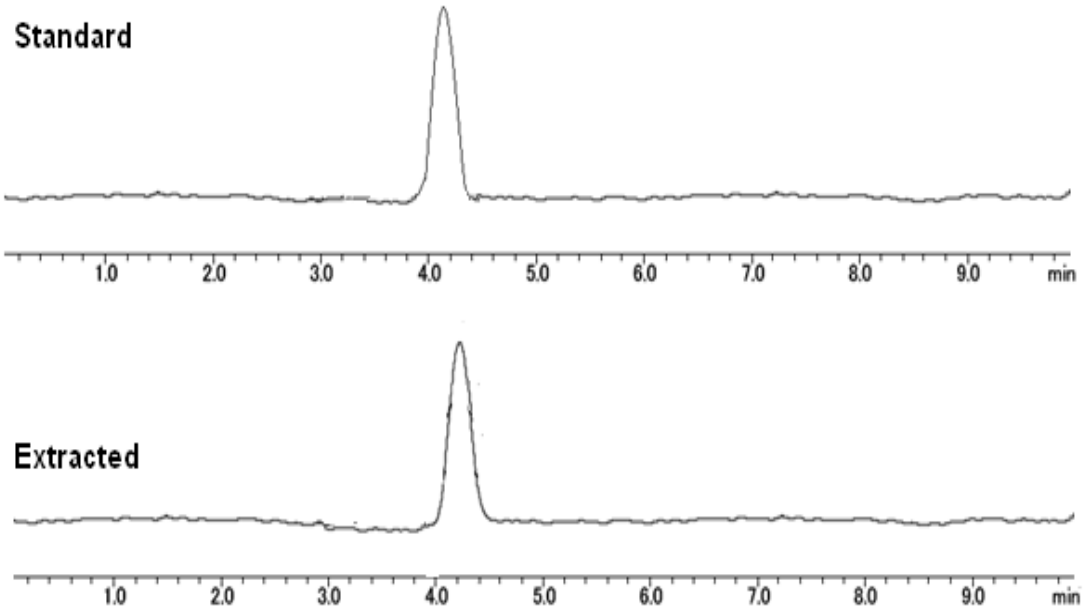

Figure 13. HPLC Standardization of β-carotene

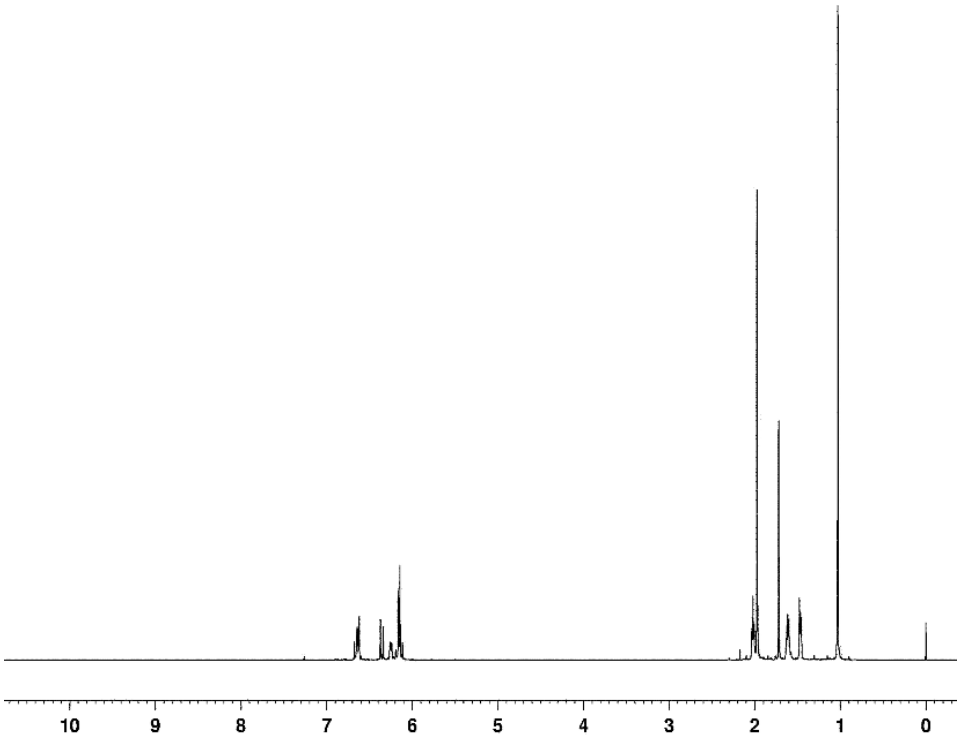

Figure 14 A. NMR Characterization of β-carotene

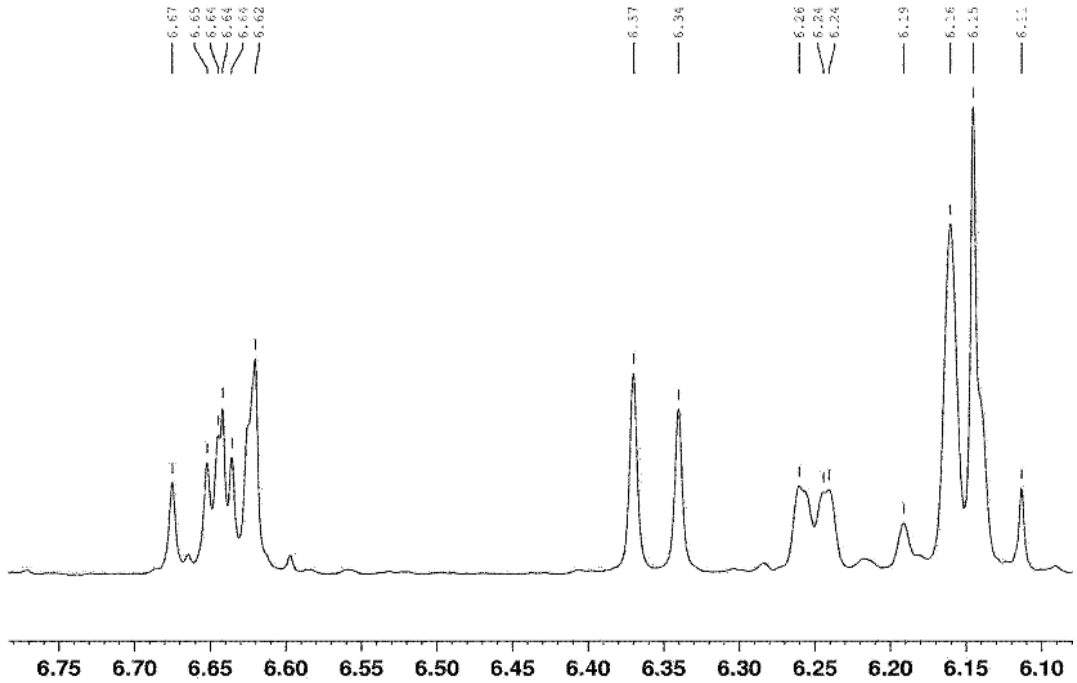

Figure 14 B. NMR Characterization of β-carotene

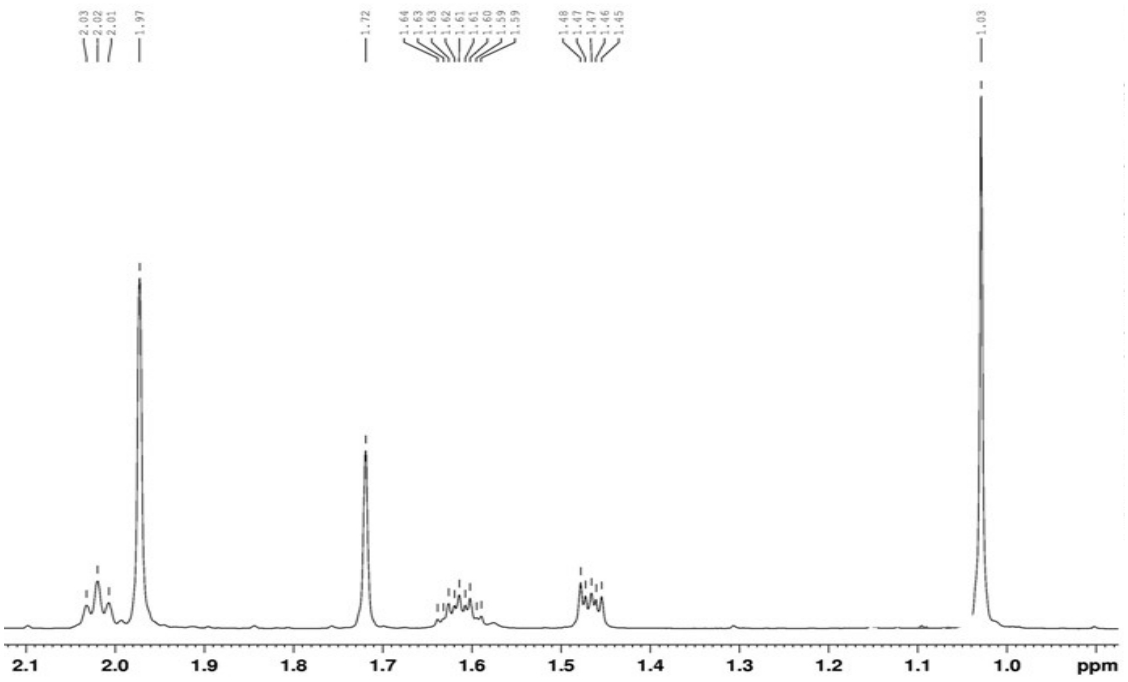

Figure 14C. NMR Characterization of β-carotene

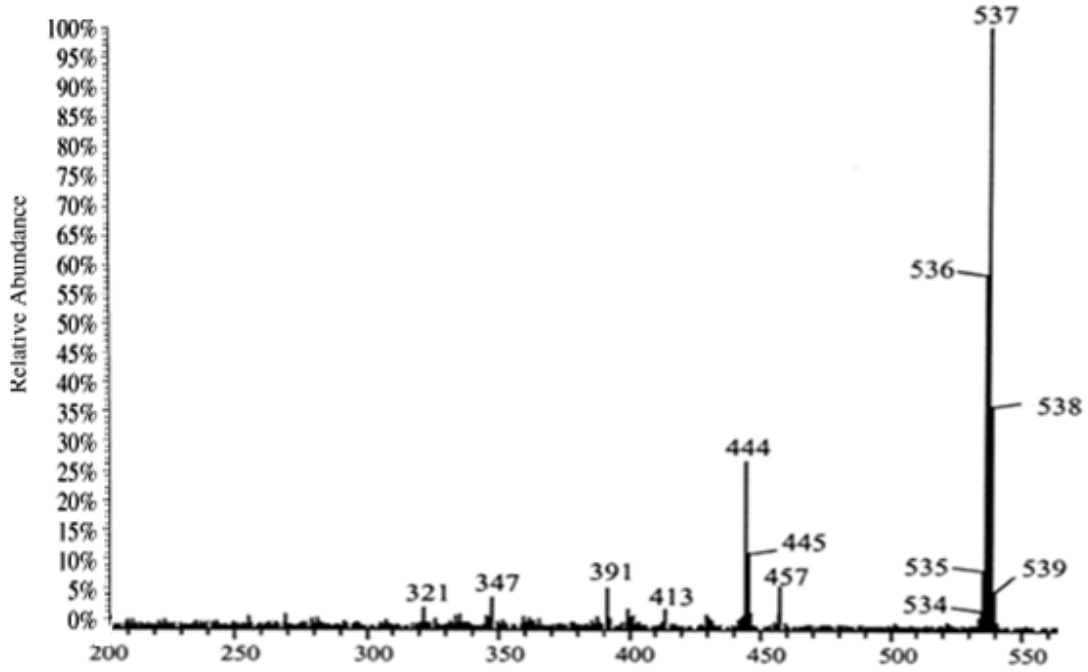

Figure 15 Mass Characterization of β-carotene

4. DISCUSSION

Piperine was extracted and isolated from 2 kg dried *piper nigrum* fruits via using the classical modified method.[7] Piperine has shown practical yield of 8.6 grams which was 0.43% of the total dried mass, sharp melting point of range 130 - 131°C has shown a purity sign. Its λ_{max} peak was matched with the standard 343 nm wavelength via UV absorption spectroscopy and found almost uniform, extracted FTIR spectra has shown similar pattern % transmittance as

reference. In HPLC the retention time of both the standard and extracted have shown the same value of 4.2 minutes in mobile phase of methanol: water at flow rate flow rate: 1ml/min at wavelength: 343 nm, NMR doublet peaks of 1.58 ppm has shown the presence of 6H ($3\times CH_2$) of piperidine atom number 18, 19, 20, peak 3.58 ppm singlet peak confirmed 4H ($2\times CH_2$) of piperidine atom number-17, 21, singlet peak of 5.96 ppm has confirmed 2H (CH_2) of dioxolane atom number 2), doublet peak at 6.42 ppm with high coupling constant of J=14.4 Hz has confirmed the a proton of transene atom number-13, doublet peak of 6.74 ppm of coupling constant J=8.4 Hz confirmed two aromatic protons atom number-6,8, doublet peak of 6.78 ppm of coupling constant J=12.8 Hz confirmed the other transene proton atom number-11 whereas doublet peak of 6.87 ppm with coupling J=7.2 Hz had confirmed another ene proton atom number-10, doublet peak at 6.97 ppm of J=1.2 Hz represents another Aromatic proton atom number-9), other transene proton at atom-12 was confirmed by double doublet peak at 6.42 ppm at coupling constant J=14.4 Hz. Mass spectra has confirmed the molecular mass of piperine by parent molecular ion peak of *m/z*: M^+ 285.2 (96%). On the basis of all the spectral and physiochemical data Piperine was standardized and characterized and its chemical structure was confirmed to the following structure.

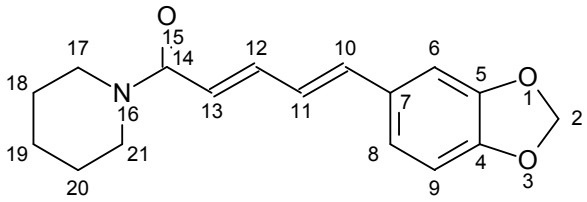

Figure 16. Piperine

Curcumin was extracted from 2 kg of dried rhizome of *Curcuma longa* and have given yield 0.60 g which was about 0.03% of total dried mass, Extracted Curcumin has shown a sharp range of melting point ranging 185-187 °C confirming its purity, It was further standardized by comparing its λ_{max} with the λ_{max} of the reference in UV absorption spectroscopy at 423 nm wavelength, in FTIR the % transmittance pattern of Extracted peaks matches with the standard, HPLC standardization was done using mobile phase: Aqueous sodium acetate buffer (0.04 M); pH=3: Acetonitrile (v/v) extracted have shown the same retention time as the reference of 7.2, mobile at flow rate of 1.0 mL/min; and at wavelength of 425 nm, NMR characterization has confirmed triplet 3.31 ppm of two protons of methylene at atom proton number 14, singlet peak at 3.91 of six protons of two methoxy group atom number 8, 26, singlet at 4.87 of two protons of two hydroxyl atom number-9,27, doublet at 6.58 of two protons having coupling constant of 14.4 Hz- confirms the transene proton atom-10,18), another doublet of 6.81ppm of two protons having coupling constant of 10.0 Hz confirmed Aromatic proton atom-4,24, coublet peak at 7.10 ppm of two protons of coupling constant 4.8 Hz confirmed the Aromatic protons atom-3,23, singlet at 7.21 ppm shown two aromatic protons atom-6,20), a doublet at 7.55 of two protons of high coupling constant of 14.4 Hz confirmed the transene protons atom-11, 17. Mass spectra confirmed the molecular weight by (M+1) *m/z*: 369.1 (38.2%). On the basis of all the spectral and physiochemical data Curcumin was standardized and characterized and its chemical structure was confirmed to the following structure.

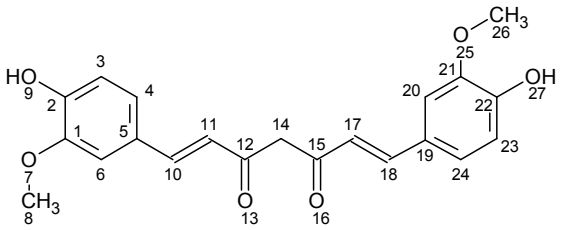

Figure 17. Curcumin

β-Carotene was also extracted and isolated by the reported and modified method from 3 kg of dried mass of *Daucus carota* roots. The yield was good of 1.256 gms which was 0.041% of the total dried weight. Its sharp melting point at 181˚C has confirmed its purest form, standardization process involved of comparing λ_{max} UV absorption peaks of extracted and reference and were found almost same at 450 nm, in Infrared spectroscopy % transmittance pattern of extracted was found almost same as reference. The extracted and reference has shown the same retention time of 4.7 min in HPLC standardization when Acetonitrile: dichloromethane: methanol in ratio of 70:20:10 as mobile phase: at flow rate: 2 ml/minute using wavelength of 450 nm. NMR characterization state triple double at 1.45 ppm of 4H having coupling constant 4, 4, 4 Hz confirmed two CH_2group atom number-25,34, quadrate at 1.59 ppm confirm four protons of two methylene groups atom number-26,35), singlet at 1.72 ppm of twelve protons of four methyl atom number-29,31,38,40), singlet at 1.97 ppm confirmed 18 hydrogen of six methyl group atom number-5,10,16,21,28,36, double doublet at 2.01 represented 4H having coupling constant of 4, and 4-Hz of two methylene atom number-24,33), range of multiplets found ranging 6.11-6.65 ppm represent fourteen protons of conjugated double bonds protons having coupling constants viz 4, 4, 8, 12, 4, 4, 4, 7 Hz) Mass spectra *m/z*: 537 (100.0%), 538 (43.9%), 539 (9.1%). Have give the idea of molecular weight. On the basis of all the spectral and physiochemical data β-Carotene was standardized and characterized and its chemical structure was confirmed to the following structure.

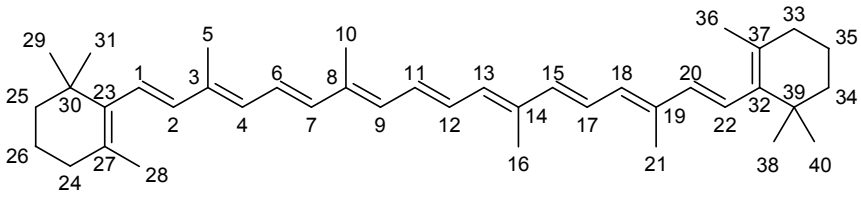

Figure 18. β-Carotene

5. CONCLUSION

On the application of the modified reported techniques, Piperine, Curcumin, and β-Carotene were extracted to their purest form from their respective sources. Based on their Physico-chemical spectral data their structures were standardized and characterized.

6. ACKNOWLEDGMENT

The authors are thankful to the Jamia Hamdard, New Delhi and Taif University, KSA for providing HPLC, NMR, IR and MASS Facility, One of the authors (MY) is thankful to UGC, for providing Fellowship.

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Conflict of Interest Reported: None Reported; Source of Funding: UGC, New Delhi, Govt. of India