



Isolation, spectroscopic characterization and molecular modeling studies of mixture of curcuma longa, ginger and seeds of fenugreek

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Abstract

Members of the Zingiberaceae such as turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* Rosc.) and fenugreek accumulate at high levels in their rhizomes important pharmacologically active metabolites that appear to be derived from the phenylpropanoid pathway. The major constituents of these spices are in ginger, the gingerols, in turmeric these are the curcuminoids and in fenugreek 5, 7-dihydroxy-2-(4-hydroxyphenyl)-6-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)chroman-4-one, diosgenin. The compound has been synthesized from the mixture of curcuma longa rhizome, ginger and seeds of fenugreek. The novel compound having chemical name is 2-(5-(3,5-dihydroxy-6-(4-hydroxy-2-(methylperoxyamino)tetrahydro-2H-pyran-3-yloxy)-4-phenyltetrahydro-2H-pyran-2-yloxy)-2-(methylperoxyamino)-6-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yloxy)tetrahydro-2H-pyran-3-yloxy)-5-hydroxy-6-methyl-4-(methylperoxyamino)dihydro-2H-pyran-3(4H)-one. The compound is characterized by various spectroscopic techniques. The compound has triclinic crystal system and the molecular structure has been optimized by MM2 calculation.

Key-words :Curcuma, Rhizome, Ginger, Shoagol, Fenugreek, Spectroscopy, Molecular Modeling

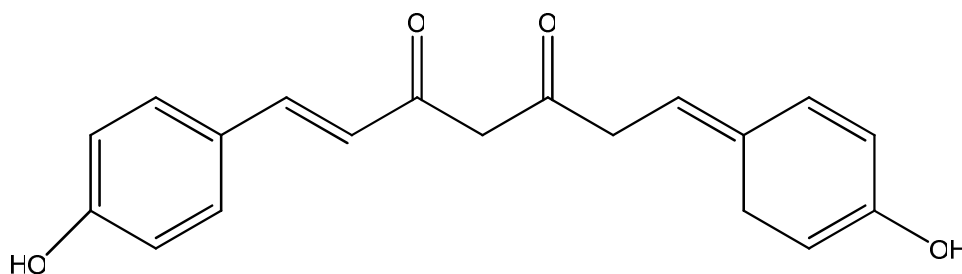
Introduction

Turmeric, ginger and fenugreek are very important spices for cooking vegetarian as well as non-vegetarian foods in Indian subcontinent. These spices are common food adjuncts that impart color, flavor and aroma. *Curcuma longa*, commonly known as turmeric is also widely used as coloring agent and known for its medicinal properties¹⁻⁵. Many research studies have been conducted on *Curcumalonga* L. (turmeric), in particular with regard to

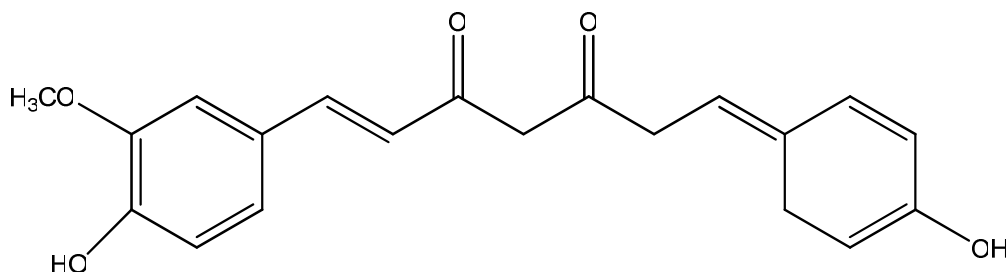
its secondary metabolites as sources of antioxidants^{6,7}. However, there is no recorded data pertaining to the abundance and quality of curcuminoids from the turmeric grown in Indian subcontinent⁸⁻¹⁰. On the basis of emerging scientific data on turmeric from various parts of the Indian subcontinent with regard to its therapeutic values¹¹, we have decided to establish oleoresin yields and the chemical fingerprints for the turmeric grown in Indian subcontinent. The aforementioned paucity in the literature is resolved as a result of the data generated from research work performed on the turmeric grown in India. *Curcuma longa* L. is a perennial rhizomatous erect herb from the Zingiberaceae family that belongs to the class Monocotyledons¹³⁻¹⁶. Some of the therapeutically active compounds in the oleoresin extracted from the rhizomes of *C. longa* are called curcuminoids. Curcuminoids are inherent compounds of the species *C. longa* and are responsible for the antioxidant activity of the oleoresin¹⁷. There are three main compounds of this pigmented curcuminoid complex, namely, curcumin chemically name is [(*E,E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], demethoxycurcumin [feruloyl(4-hydroxycinnamoyl)methane], and bisdemethoxycurcumin or bis(4-hydroxycinnamoyl)methane (Figure 1). Curcumin (C₂₁H₂₀O₆) constitutes 50–60% of the curcuminoids found in *C. longa*. In addition to the curcuminoids, there are essential oils containing monocyclic monoterpenes, sesquiterpene (bisabolanes and germacranes), arabinogalactans and *ar*-turmerone¹⁸. The confluence of healthy living and eating has been the impetus for the scientific disciplines of functional foods and nutraceuticals¹⁹. Although there have been many characterization studies of the turmeric rhizome²⁰, there is still uncertainty with regard to the effects of extracting freshly processed rhizomes versus dried milled rhizomes on the quantity and quality of their oleoresins. The phenolic secondary metabolites, curcuminoids, play an important role in the quality of turmeric-containing foods because they affect the organoleptic traits of the foods in terms of aroma, flavor, and taste. The stability of curcuminoids, their biosynthesis, and degradation are influential to food quality. In terms of biosynthesis, one of the key enzymes, phenylalanine ammonia-lyase, can be induced by different environmental stress conditions²¹. Appropriate post harvesting processing of the rhizome can sustain phenolic quality of plant material²². These novel scientific findings will no doubt build leverage for the turmeric grown in Indian subcontinent in the marketplace, benefiting the various Indian spice houses and commercial exporters of the spice. Ginger (*Zingier officinale* Roscoe; family Zingiberaceae) is a monocotyledonous, sterile cultigen thought to have originated in India or Southeast Asia, from where it was introduced to other parts of the world^{26,27}. Both fresh and dried ginger rhizomes are used

worldwide as a spice, and ginger and ginger extracts are used extensively in the food, beverage, and confectionary industries in the production of products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, biscuits, and other bakery products. The unique flavor properties of ginger arise from the combination of pungency and aromatic essential oil. Ginger is also widely used in both traditional and contemporary natural medicine. It has been used medicinally in India since ancient times and is mentioned in Vedic texts dating back to around 4000 BCE²⁸. Ginger is included in the British, European, Chinese, and Japanese pharmacopoeias, as well as in many other national pharmacopoeias, and the World Health Organization has published a monograph for *Rhizoma Zingiberis*. The medicinal uses of ginger are diverse and include the treatment of dyspepsia, colic, diarrhea, colds and flu, and poor appetite. It is also recommended as an anti-inflammatory agent in rheumatic and muscular disorders and to increase longevity²⁹. Clinical trials support the use of ginger preparations in the prevention of motion sickness³⁰. and vomiting in pregnancy³¹, while the evidence is more ambiguous in the case of musculoskeletal disorders. The main pungent compounds in fresh ginger are a series of homologous phenolic ketones known as gingerols (Figure 3). The gingerols are thermally unstable and are converted under high temperature to shogaol (after *shoga*, the Japanese word for ginger;) . Shogaols, which are more pungent than gingerols, are the major pungent compounds in dried ginger .The active ingredient in turmeric is curcumin and that in ginger are gingerol and hexahydrocurcumin Both these compounds prevent oxidation of oils and fats. *Trigonella foenum graecum* Linn belongs to the family Leguminosae and it is popularly known as Fenugreek³². *Trigonella foenum graecum* Linn is native to the area from Indian subcontinent and also much cultivated in India and China. Fenugreek is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicines³³. In India, the seeds of fenugreek were used in Ayurveda and Siddha to treat fever, dysentery heart diseases and diabetic while in Unani system, this plant issued as a resolvent, aphrodisiac, diuretic, emmenagogue and tonic. In China, fenugreek seed swear used as a galactogogue to encourage lactation³⁴. The past phytochemical investigations on the seeds reveals the presence of Diosgenin, Trigonelline, Gitogenin, Vicenins 1 and 2, Vitexin, Quercetin, Luteolin, Kaempferol, Sitosterol etc., moreover the endosperm of the seeds is rich in galactomannan..The major constituents of fenugreek are given in figure. Several synthetic spasmogens and spasmolytics have been used in the field of medicine³⁶ [, in spite of their side effects and minimum therapeutic index. Even though many drugs are available with spasmogenic and spasmolytic properties, search for a drug of plan origin

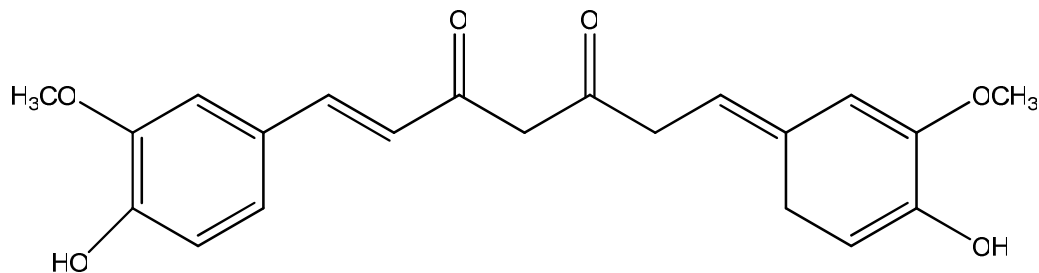
with maximum potency and minimum side effects continues. Since no scientific report of the previous investigations detail are available on the effect of fenugreek on the isolated smooth muscles, the present study was undertaken to screen the isolation mixture of curcuma rhizome, ginger and seeds of fenugreek and characterized by physical techniques.



(1E,7E)-7-(4-hydroxycyclohexa-2,4-dienylidene)-1-(4-hydroxyphenyl)hept-1-ene-3,5-dione

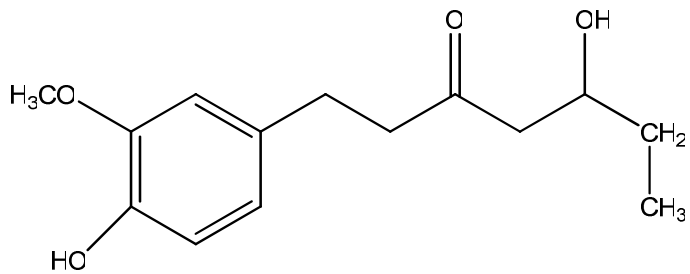


(1E,7E)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxycyclohexa-2,4-dienylidene)hept-1-ene-3,5-dione

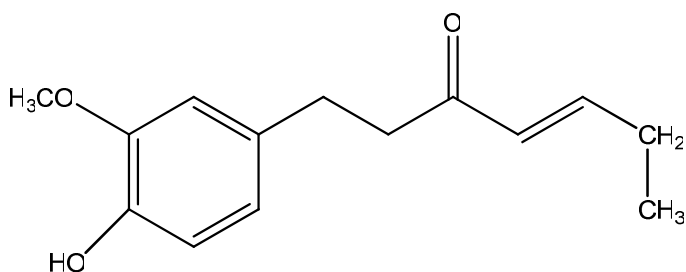
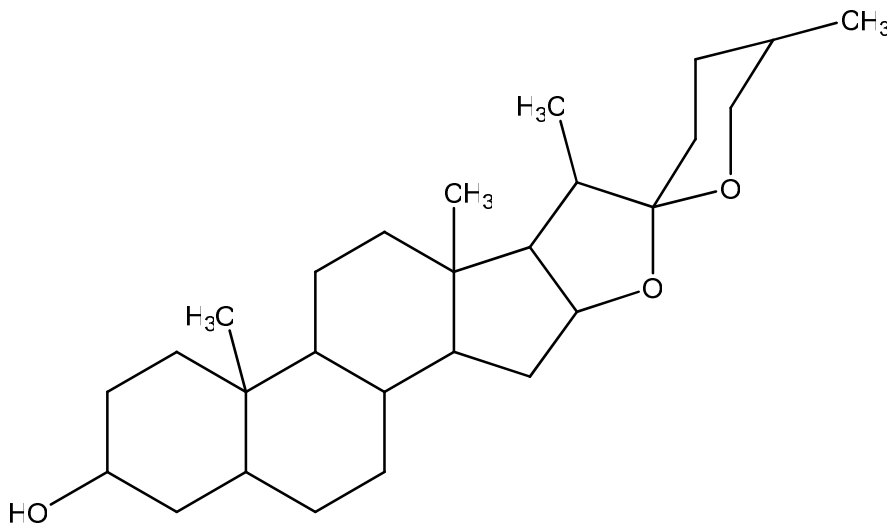


(1E,7E)-7-(4-hydroxy-3-methoxycyclohexa-2,4-dienylidene)-1-(4-hydroxy-3-methoxyphenyl)hept-1-ene-3,5-dione

Figure1 Three curcuminoid structure



5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)heptan-3-one

*(E)*-1-(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one**Figure 2: Structures of the major pungent compounds in ginger, gingerol and shogaols.****Figure 3. Structure of diosgenin.**

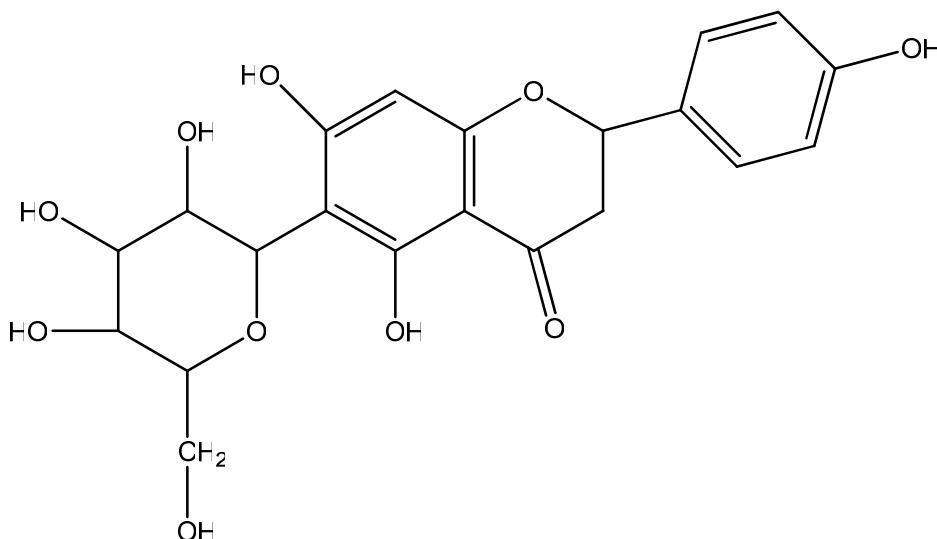


Figure4..5,7-dihydroxy-2-(4-hydroxyphenyl)-6-(3,4,5-trihydroxy-6(hydroxymethyl) tetrahydro-2H-pyran-2-yl)chroman-4-one

The mixture of tumeric, ginger and seeds of fenu greek generally used in joint pain relief in rural area of subcontinent as well as heart disease. The aim of this paper is to isolate a mixture of the spices and spectroscopy studies of isolated compound. On the basis of experimental observation the structure of the proposed compound can be established.

Experimental

Material and Methodology

All the chemicals used were of analytical grade. The stoichiometric analyses (C, H and N) of the product performed using Elementar vario EL III (Germany) model. Their IR spectra were recorded on Perkins–Elmer FTIR spectrophotometer in KBr and polyethylene pellets. The electronic spectra were recorded in water on Beckman DU-64 spectrophotometer with quartz cells of 1 cm path length and mass spectra (TOF-MS) were recorded on Waters (USA) KC-55 model with ES⁺ mode in D₂O. ¹H NMR spectra were recorded in CDCl₃ solvent on a Bruker Advance 400 instrument. Rigaku model 8150 thermoanalyser (Thermafex) was used for simultaneous recording of TG-DTA curves at a heating rate of 5^omin⁻¹. For TG, the instrument was calibrated using calcium oxalate while for DTA, calibration was done using indium metal, both of which were supplied along with the instrument. A flat bed type aluminium crucible was used with α-alumina (99% pure) as the reference material for DTA. The activation energy and Arrhenius

constant of the degradation process was obtained by Coats and Redfern method³⁷. The XRD powder pattern were recorded on a vertical type Philips 1130/00 x-ray diffractometer, operated at 40kV and 50Ma generator using the Cu α line at 1.54056Å as the radiation sources. Sample was scanned between 5° to 70°(2 θ) at 25°C. The crystallographic data was analyzed by using the CRYSFIRE-2000 powder indexing software package and the space group was found by the CHECKCELL programme. Debye-Scherrer relation with the help of 100% peak width determined the particle size. The density was determined using conventional Archimedes method.

3D - Molecular Modeling

Molecular modeling was performed by the latest version of the software. 3D molecular modeling of the proposed structure of the compound was performed using CsChem3D program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The potential energy of the molecule was the sum of the following terms: $E = E_{\text{str}} + E_{\text{ang}} + E_{\text{tor}} + E_{\text{vdw}} + E_{\text{oop}} + E_{\text{ele}}$. Where all E's represent the energy values corresponding to the given types of interaction. The subscripts str, ang, tor, vdw, oop and ele denote bond stretching, angle bonding, torsion deformation, van der waals interactions, out of plain bending and electronic interaction, respectively.

Synthesis of mixture of Curcuma Longa, Ginger and Seeds of Fenugreek

Turmeric rhizome, ginger and Fenugreek seeds were obtained from the local market of Delhi, India. The rhizomes, ginger and seeds fenugreek were washed with methanol cleaned, dried in air oven at 60 ° C then grind to a powder using an electric grinder to pass a 0.4 mm screen. The powder was taken in a cleaned round bottom flask contained methanol then stirred 12h. The solution was filtered and washed with hot methanol. The above product was redissolved in excess warm methanol, and clear solution was left undisturbed for weeks to give beautiful crystals were obtained. Various attempts to obtain the single crystals have so far been unsuccessful.

Grey crystal; yield: 80%; anal. calcd. for $C_{36}H_{57}N_3O_{24}$ (M.P.⁰C 25.5) requires(%): C,47.21; H, 6.27; N,4.59. Found: C,47.25; H, 6.45; N,4.56; FTIR(KBr, cm^{-1}): ν (OH) 3410(s, b) and 527(w), ν (NH)3365(s, b) and 2926(m), ν (C=O)1656(s), ν (-

C-O)1025(m), ^1H NMR(400MHz, CDCl_3 ,ppm) δ 3.38-3.64(m,8H), 2.0(m,3H),3.09-5.03(m,8H),3.39 and 1.18(d,9H). UV(nm): 428 ($n \rightarrow n^*$),240-250($n \rightarrow \pi^*$), TG[Step I, Step II] E^* (J mole^{-1}): 31.28,32.86; A ($\times 10^4 \text{sec}^{-1}$): 0.36,0.28 ; ΔS^* ($\text{JK}^{-1}\text{mol}^{-1}$): - 161.43, - 167.25; ΔH^* (J mole^{-1}): 53.24, 78.07 and ΔG^* (kJ mol^{-1}): 78.99, 93.69]; DTA[Step I, Step II,]: Exo, Exo;

Results and Discussion

Satisfactory results of elemental analysis and spectral studies revealed that the compound was of good purity. X-ray diffraction studies indicate crystalline nature of the isolated compound.

Spectral characterization

The IR spectrum of the compound has been studied in order to elucidate its structure. In the spectrum, the presence of broad bands at 3410 and 3365 cm^{-1} can be attributed to ν (OH) and ν (NH) stretching vibrations respectively. The presence of strong to medium intensities bands were also observed at 2926 cm^{-1} which confirms N-H stretching frequency [38](Figure5). Other strong to medium intensity bands were also observed at 1656 cm^{-1} presence of C=O group and 1411 – 1162 cm^{-1} due to the presence of the phenyl group. Some other band appeared at 1025 and 527 cm^{-1} presence of –C-O and OH group.

The ^1H NMR spectra of the synthesized compound show well-resolved signals appeared. The compound show the resonance with integrated intensities (Figure6). The chemical shift of the compound appeared at 3.38---3.64(8H,m)for OH-alcohol,2.0(3H,m) for NH,amine, 3.09-5.03,(8H, m) for CH, 7.27-7.30(5H,m) for CH of benzene,3.39 and 1.18(9H,d,) for CH_3 .

The electronic spectra (UV-Vis) of the compound have been studied in the range 190 – 800 nm. The shoulder band observed at 428 nm in compound may be assigned to $n \rightarrow n^*$ transition within the C=O group of the carbonyl group. A strong band in the region 240-250nm was observed may be due to $n \rightarrow \pi^*$ transition in the aromatic ring.

Mass spectrometry has been successfully used to investigate molecular species of the isolated compound in solution⁹⁻¹¹ . The molecular ion peak of the compound has been

used to confirm the proposed formula (Figure 7). The pattern of the mass spectrum gives an impression of the successive degradation of the target compound with the series of peaks corresponding to the various fragments. Their intensity gives an idea of stability of fragments. The first two fragments appears at 897/899/913/915(10%*m/z*) and 526(85% *m/z*) corresponds to $[C_{36}H_{57}N_3O_{24}]^+$ and $[C_{19}H_{32}NO_{16}]^+$ respectively, which could be the result of degradation of the compound. The compound after degradation, finally forms $[C_{13}H_{24}N_2O_{10}]$, 368/369(50% *m/z*). The extra stability of *m/z* values at 526 and 687 may be because of intermolecular hydrogen bonding.

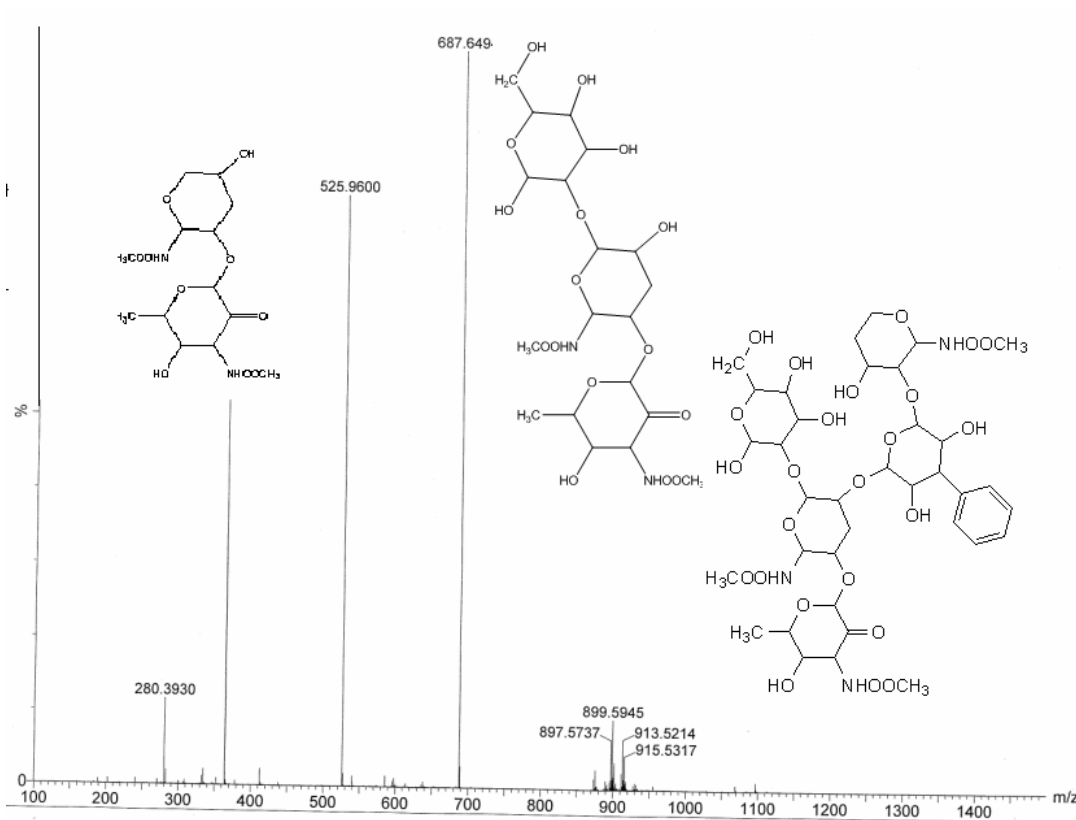


Figure 7: TOF-Mass spectra of the compound

Kinetics of thermal decomposition

Recently, there has been increasing interest in determining the rate- dependent parameters of solid-state non- isothermal decomposition reactions by analysis of TG curves^{25,26} . Thermogravimetric (TG) and differential thermo gravimetric (DTA) analyses were carried out for the compound in ambient conditions. The thermogravimetric analysis revealed that the compound was entirely decomposed to 400°C. On the basis of thermal decomposition, the kinetic analysis parameters such as activation energy (E^*), enthalpy of activation (ΔH^*), entropy of activation (ΔS^*), free energy change of decomposition (ΔG^*) were evaluated graphically by employing the Coats – Redfern relation³⁷ to give linearization of curve.

$$\text{Log} [-\text{Log} (1 - \alpha) / T^2] = \text{log} [AR / \theta E^*(1 - 2RT/E^*)] - E^*/2.303RT$$

Where α is the mass loss up to the temperature T , R is the gas constant, E^* is the activation energy in J mole^{-1} , θ is the linear heating rate and the term $(1 - 2RT/E^*) \cong 1$. A straight line plot of left hand side of the equation (1) against $1/T$ gives the value of E^* while its intercept corresponds to A (Arrhenius constant). The Coats and Redfern linearization plots, confirms the first order kinetics for the decomposition process in two steps²⁸ . The thermodynamic activation parameters for the decomposition steps have been calculated that relates the thermal stability of the compound. The negative value of entropy also indicates a more ordered activated state that may be possible through the chemisorptions of oxygen and other decomposition products. The negative values of the entropies of activation are compensated by the values of the enthalpies of activation, leading to almost the same values for the free energy of activation.

X-ray powder diffraction studies

In absence of single crystal, X-ray powder data is especially useful to deduce accurate cell parameters. The diffraction pattern reveals the crystalline nature of the complex. The indexing procedures were performed using (CCP4, UK) CRYSFIRE. From the crystallographic data the compound having triclinic crystal system and space group P_1 as well as particle size is 11.4587nm. The detail crystallographic data are given in table 1.

Table1. Crystallographic data of the compound

Empirical Formula	C ₃₆ H ₅₇ N ₃ O ₂₄
Formula weight	915.84
Temperature (K)	298
Wave length (Å°)	1.54056
Crystal system	Triclinic
Space group	P ₁
Unit cell dimensions (Å°)	a=5.092227 b=7.836355 c=8.793142 α=43.745970°, β=85.905460 γ=82.250500°
Volume (Å ³)	1240.24
2θ Range	10° - 70°
Limiting Indices	-2 ≤ h ≤ 2 -1 ≤ k ≤ 4 0 ≤ l ≤ 5
Density (g/cc)	1.22
Z	1
Avs. Eps	0.0000976

Molecular structures & analysis of bonding modes

To examine the structural properties, various traditional research techniques were used, we were trying to assess observed data at molecular level with the help of molecular modeling. This modeling program was commonly known as computer assisted molecular design (CAMD). Molecular modeling had been successfully used to detect three dimensional arrangements of atoms in isolated compound. Their utilization in the demonstration of molecular structure of the studied compound was presented in the article. Molecular mechanics was a mathematical formalism, which attempted to reproduce molecular geometries, bond energies and other related features. Bond lengths, bond angles and atomic coordinates and their values were dependent on the hybridization of an atom and its bonding scheme³¹⁻³⁵. The optimized structure was obtained by using

of Chemoffice Ultra-11 software programme. The optimized energy of the compound is 65kJ/mol (Figure 8) The structure was performed by given structure and obtained data through density functional theory. The minimum energy configurations support the proposed structure.

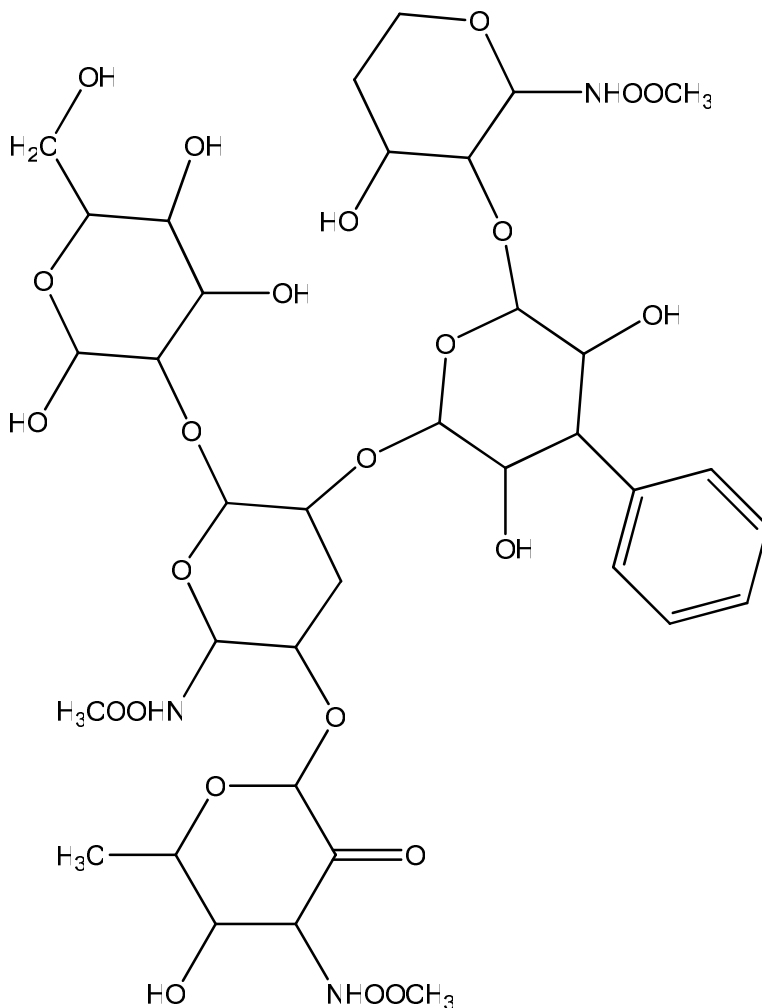


Figure11. Structure and chemical name of synthesized compound 2-(5-(3,5-dihydroxy-6-(4-hydroxy-2-(methylperoxyamino)tetrahydro-2H-pyran-3-yloxy)-4-phenyltetrahydro-2H-pyran-2-yloxy)-2-(methylperoxyamino)-6-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yloxy)tetrahydro-2H-pyran-3-yloxy)-5-hydroxy-6-methyl-4-(methylperoxyamino)dihydro-2H-pyran-3(4H)-one

Chemical Formula: C₃₆H₅₇N₃O₂₄

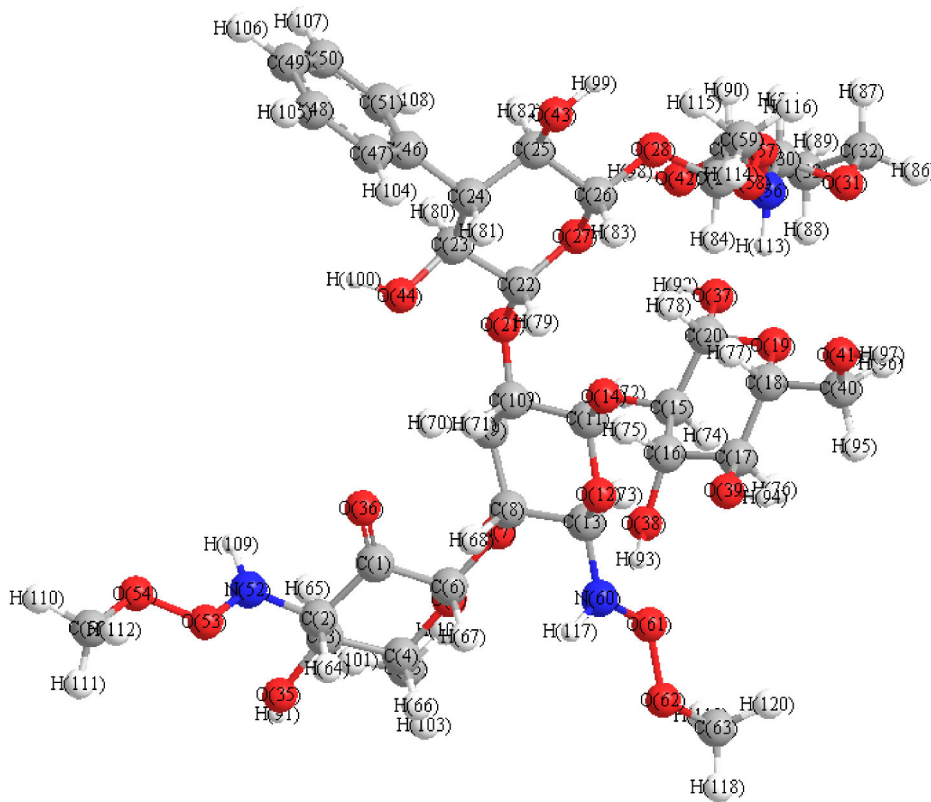


Figure 8: Optimized Structure of the compound

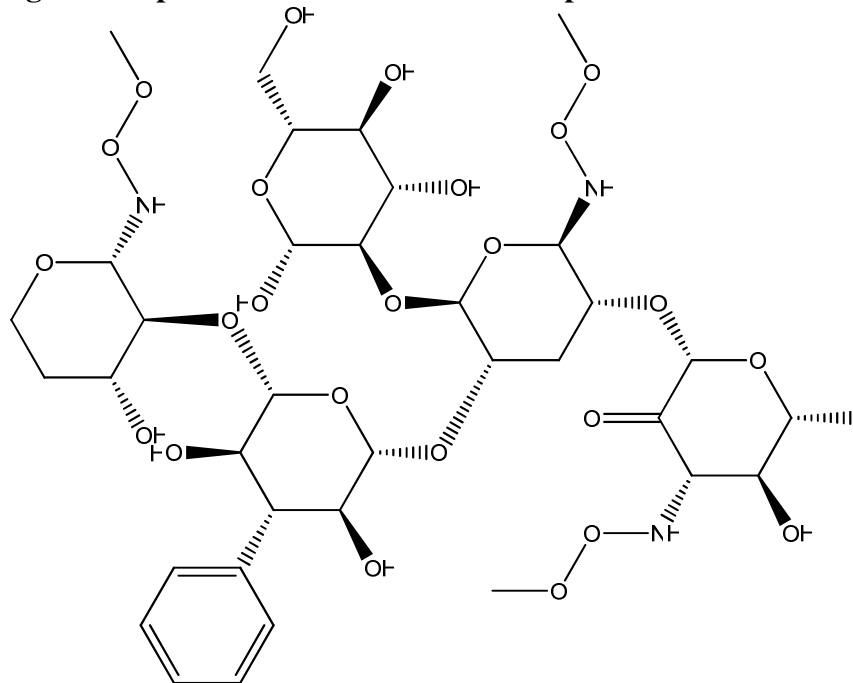


Figure10.Stereostructure

The stereo structure (figure 10) show that the molecule contains chiral carbon indicated that asymmetry character.

Conclusion

From the elemental analysis the empirical formula of novel compound (Figure 11) is $C_{36}H_{57}N_3O_{24}$. It is characterized by various spectroscopic techniques (FTIR, 1H NMR, UV, Mass), TGA/DTA and XRPD studies. The molecular modeling has been used for the optimization of the structure of the compound. This compound will be used in medicine as complex type of medicine because in Indian subcontinent the mixture of turmeric, ginger and fenugreek in powder form being used traditionally for the treatment of pain killer, heart disease and anti diabetic.

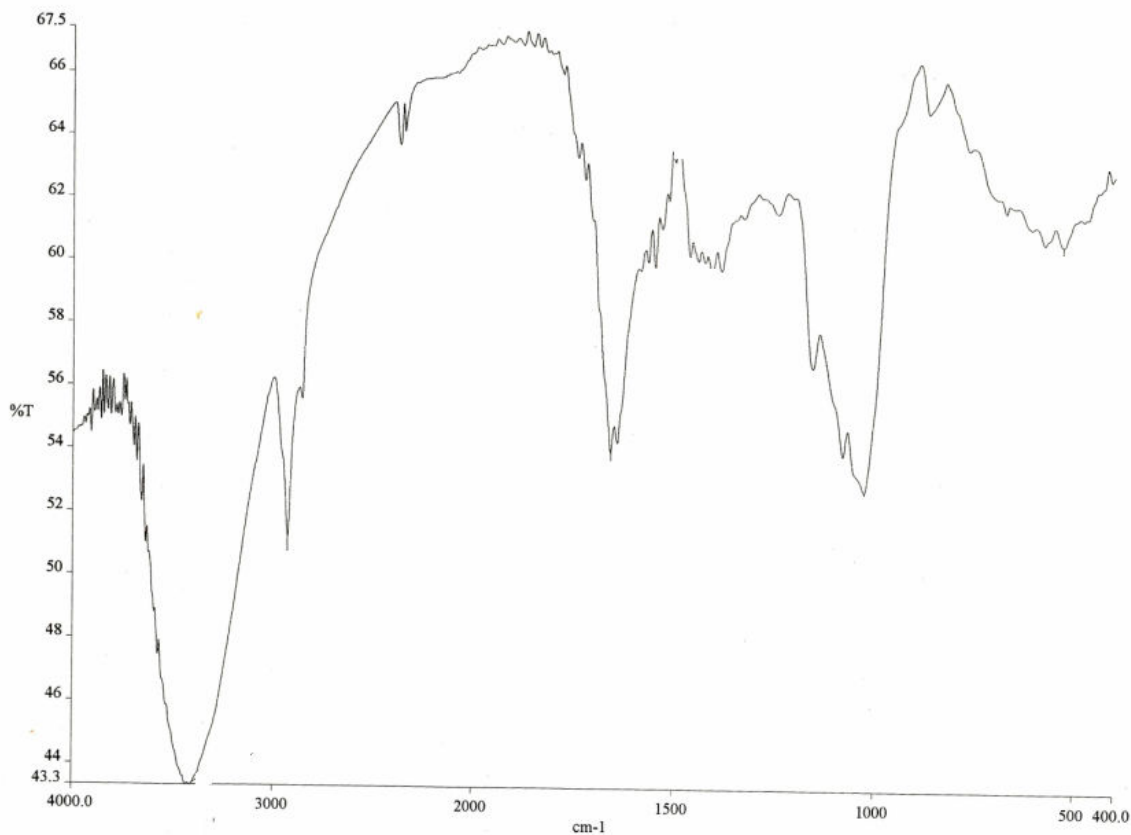


Figure5: IR spectrum of the compound

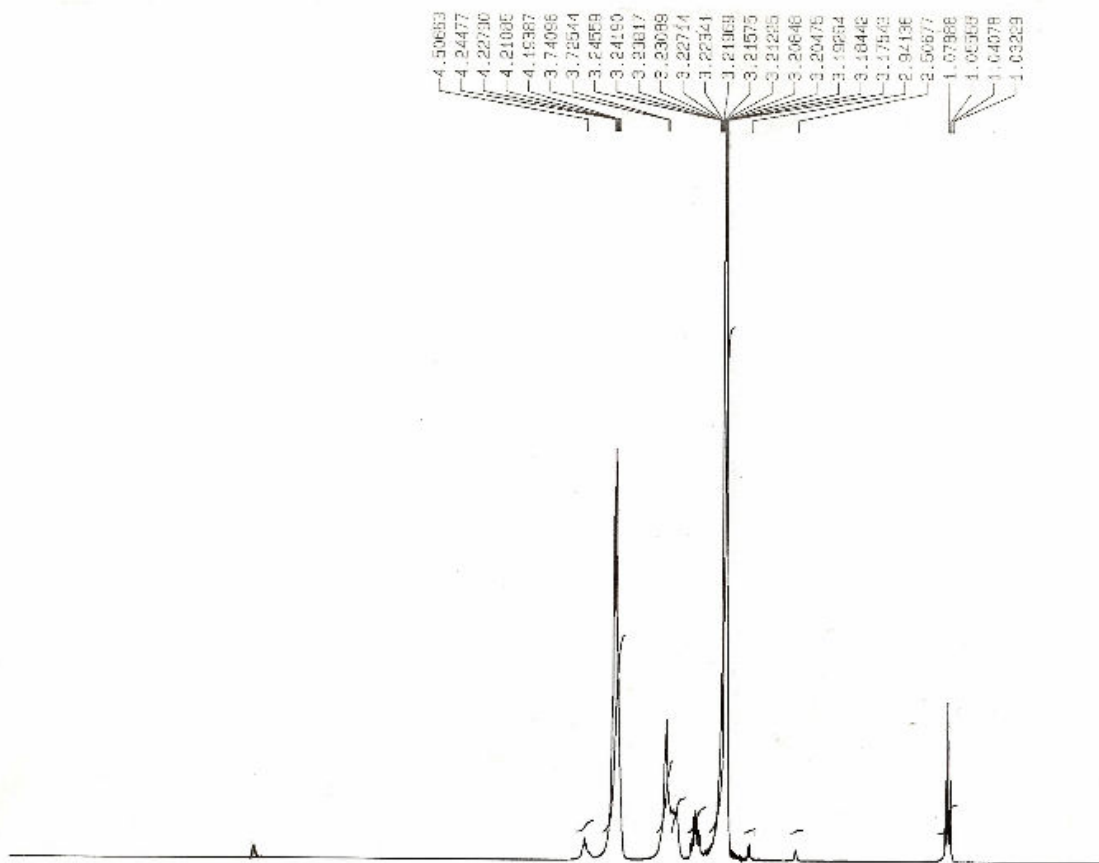


Figure6: ¹H NMR spectra of compound

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