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SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT ACTIVITY AND ANTICANCER ACTIVITY OF L-PHENYL ALANINE -BIS-MANDELATE

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ABSTRACT

The stress and strain experienced physically and mentally with the intake of insufficient unhealthy food give age related health problems. The unhealthy diet consumption and irregular intake of on time food habit of human beings are giving the formation of reactive oxygen species (ROS). The diseases caused by the food habits, routine stress and strain can be tackled by scavenging the ROS immediately after the formation. Small organic molecules possessing donor-acceptor functional groups are resulting in the formation of hydrogen bonding interactions which stabilises the molecular structure. The weak vander Waals forces of attraction and charge transfer mechanisms are resulting in the formation of low energy gap organic salts with high chemical reactivity and low chemical stability. These salts showed high antioxidant activity, high antiradical power and the presence anticancer property. Synthesised novel organic salt, L-phenyl alanine- bismandelate (BMALPA), the title compound, by the combination of mandelic acid (MA) and L-phenyl alanine (LPA) in the molar ratio 2:1 in the aqueous medium. The characterisation studies, determination of antioxidant activity by chemical and electrochemical methods and in vitro anticancer property using liver carcinoma cell line HepG2 and normal Vero cell line of the title compound showed its promising ability to act as an anticancer material due to the presence of increase in number of hydroxyl groups.

KEYWORDS: ros, bmalpa, antioxidant activity, antiradical power, Hep G2 cell line and Vero cell line.

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INTRODUCTION

The metabolic activities involve the production of reactive oxygen species(ROS). The presence of ROS in human body propagate as chain reaction. The stress and strain in the environmental changes increase the superoxides (ROS) formation in our body.[1] This oxidative stress induced chain reaction is nullified by the edible ingredients. [2] Improper dietary consumption enhance the oxidative stress which leads to the generation of carcinogenic diseases.[3] Small organic molecules are used as scavengers to terminate the superoxides and act as an anticancer drug[4]. The high polarisability and acidic nature of alpha hydroxy phenyl acetic acid, MA showed good dielectric property and electronic property.[5] Amino acids posses intra molecular charge transfer ability and form zwitterions.[6] The aromatic substitutions in amino acid enhance the ability of formation of zwitterions. Hence the combination of MA and LPA are tried in different molar ratios. The molar ratio 2: 1 of MA and LPA resulted in the formation of organic salt, BMALPA , the title compound. The applications of MA in the cosmetics and photo aging are already known.[7] The biological applications of essential amino acids are studied and reported.[8] The novel organic salt, the title compound analytical results , in vitro study details are discussed in this paper.

MATERIALS AND METHODS

DL-mandelic acid (Alfa Aaesar 99% pure) and L-phenyl alanine (Nice chemicals 98% pure) are used for the synthesis of title compound.

Synthesis of l - phenyl alanine bismandelate (bmalpa)

0.06 mmoles of MA is taken in a clean beaker and 1.6 mmoles of distilled water is added, stirred well at room temperature for 30 minutes. 0.03 mmoles of LPA is taken in a clean beaker and 1.6 mmoles of distilled water is added and the contents are stirred well at room temperature for 30 minutes. MA solution is added to LPA solution slowly at room temperature and the complete dissolution is observed after 30 minutes of stirring at room temperature. The solution is filtered and the clear colourless solution obtained is kept for slow evaporation at room temperature. The white crystals of BMALPA appeared after 30 days. It showed homogenity on TLC and the melting point is 173˚C. The yield is 65%.

Schematic representation of BMALPA synthesis

$$2C_6H_5CH(OH)COOH + C_6H_5CH_2CH(NH_2)COOH + H_2O$$

DL- Mandelic acid L-phenyl alanine

↓

$$2C_6H_5CH(OH)COOH.C_6H_5CH_2CH(NH_2)COOH\,.\,H_2O$$

L-phenyl alanine bismandelate (BMALPA)

The synthesised title compound is subjected to the various characterisation studies to confirm the presence of functional groups using fourier transform infra red spectrophotometer, types of linkages,the nature of protons and carbons using proton and carbon NMR, the molecular mass determination using mass analysis, the crystalline nature using XRD measurement, the light absorption and emission behaviours using photoluminescence analysis, the thermal stability at high temperature using TG-DTA analysis, antioxidant activity using chemical and electrochemical techniques and anticancer property using in vitro study.

RESULTS AND DISCUSSION

FTIR of BMALPA (Fig 1), shows hydrogen bonded NH_2 group with asymmetric stretching at 3165 cm^{-1}, -C-H stretching at 3031 cm^{-1}, 2838 cm^{-1} and 2546 cm^{-1} , $-CH_2$ symmetric stretching at 2902 cm^{-1} , C=O stretching at 1725 cm^{-1}, - NH_2 deformation at 1594 cm^{-1}, $-CH_2$ scissoring vibrations at 1451 cm^{-1} and 1421 cm^{-1}, C-N stretching at 1187 cm^{-1}, C-O-C stretching at 1160 cm^{-1} and 1105 cm^{-1}, CH-O-H stretching at 1062 cm^{-1}, carbon ring vibrations at 1032 cm^{-1} and 983 cm^{-1}, CH_2 out of plane bending at 948 cm^{-1}, C-H out of plane deformation at 862 cm^{-1}, C-N-C bending at 529 cm^{-1} and C-O-C bending at 489 cm^{-1} respectively.[9-11]

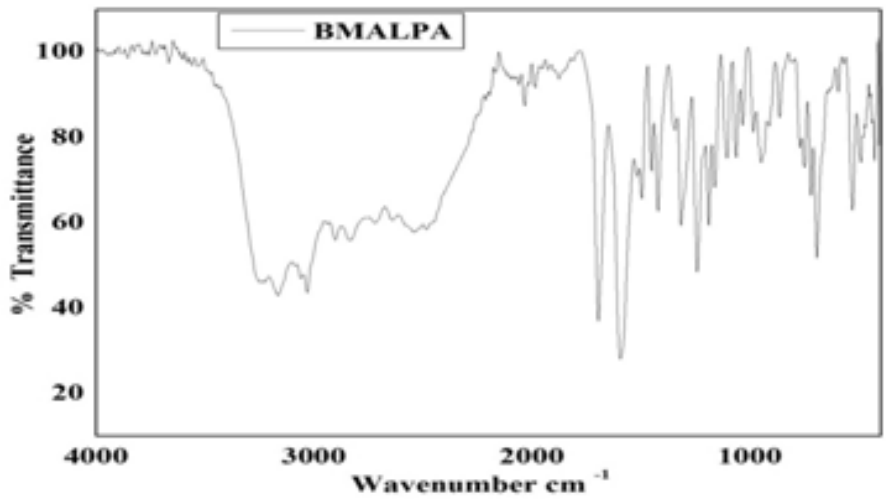

Figure 1
FTIR of BMALPA

The proton NMR and the carbon NMR of the title compound in (Fig 2) and (Fig 3) are confirming the chemical shifts due to carbon and proton linkages present in the title compound and are given in the Table 1. Electron density rich nucleus shielded protons are observed in the upfield.[12-13]

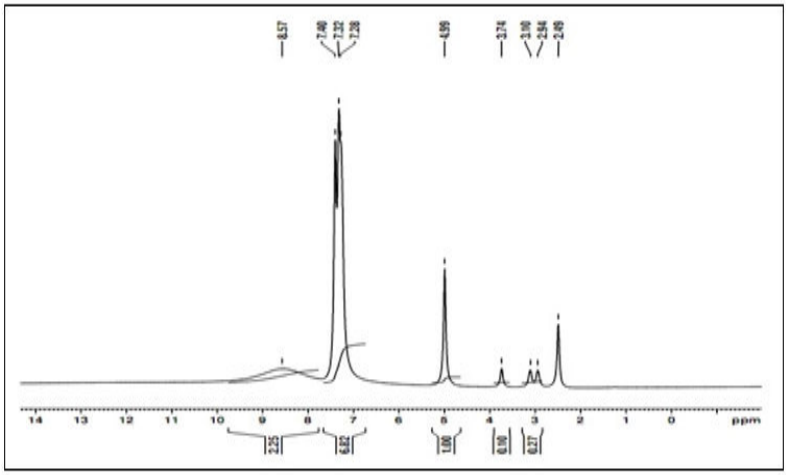

Figure 2
Proton NMR of BMALPA

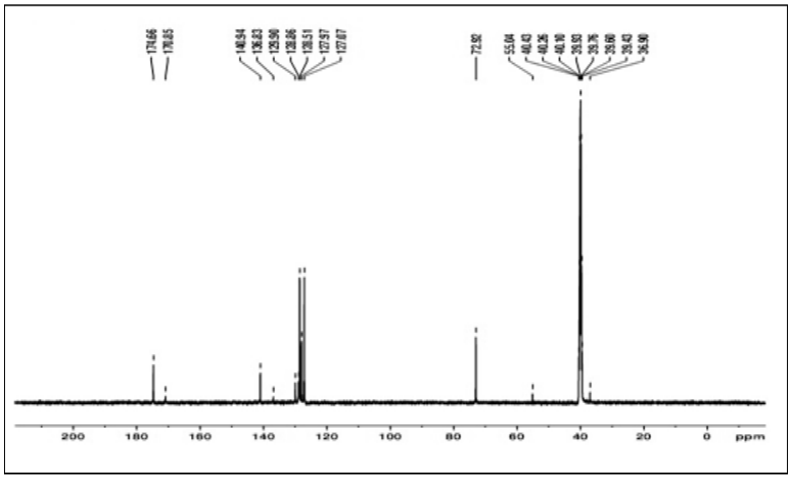

Figure 3
Carbon NMR of BMALPA

Table 1
Carbon and Proton NMR of BMALPA

Sample	^{13}C NMR	Sample	^{1}H NMR
Solvent	CD_3-SO-CD_3	Solvent	CD_3-SO-CD_3
Solvent peak	39-40	Solvent peak	2.5-2.6
C - H Carbon	36.9	N-H Proton	2.94
C-N Carbon	55	CH_2 Proton	3.1
C - OH Carbon	72.9	CH Proton	3.7
Aromatic carbons	120-140	C-OH Proton	4.99

The expected formula weight of BMALPA is 488 and the mass spectral value obtained is 490 using mass spectral analysis. The difference may be due to the ionisation and fragmentation for the title compound happening in the gaseous phase.[14-18] The results of XRD study of BMALPA crystal unit cell parameters is showing the presence of monoclinic crystal system with C2 space group having dimensions a = 19.52 Å α= 90°, b = 5.75 Å β= 114.74°, c = 15.84 Å γ = 90° and Volume 1615 Å^3. The crystal nature is unsuitable for structure resolution indicating that the compound may be having microcrystalline structure.[19] Hence BMALPA is subjected to powder XRD analysis. The powder XRD pattern of MA and LPA are compared with that of title compound. The comparison showed that the obtained product is a pure cocrystal.[20] The $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions shown in (Fig 4) from the absorption peaks at UV-Visible region, due to the presence of conjugative system and charge transfer interactions in the title compound.[21-22]

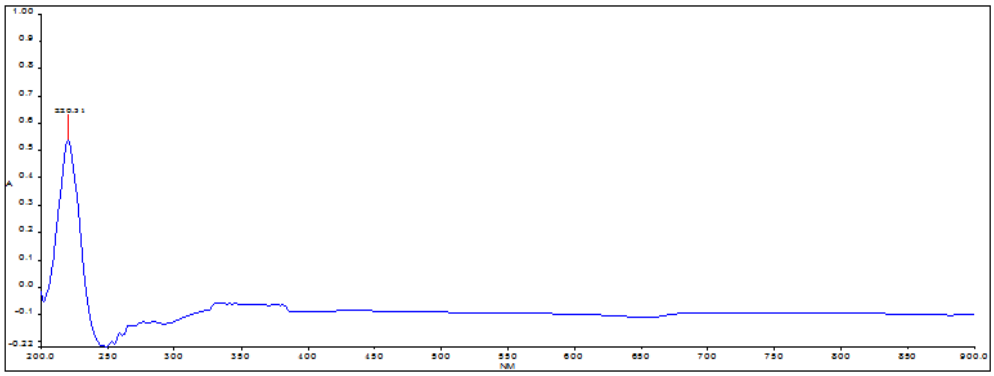

Figure 4
UV spectrum of BMALPA

The broad excitation and emission peaks shown in (Fig 5) are due to the collisions and electrostatic interactions happening in the polar solvent methanol with the title compound. Compounds containing π → π* transition levels have high intense fluorescence behaviour.[23]

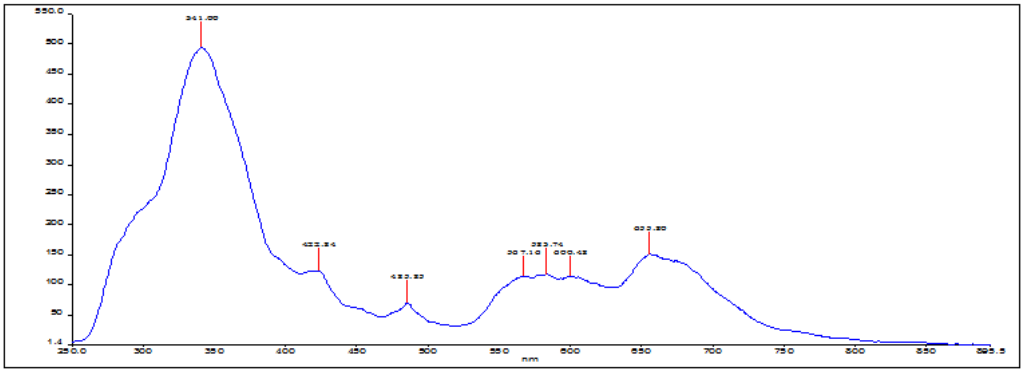

Figure 5
Fluorescence spectrum of BMALPA

TG-DTA curve of the title compound (BMALPA) in (Fig 6), is showing one endothermic peak at 184˚C without weight loss. The endothermic decomposition is starting around 200˚C and exothermic peak is observed at 351˚C. The presence of both endothermic and exothermic peaks are indicating that the thermal decomposition is followed by multistage oxidative decomposition. The actual melting point of the title compound is 173˚ C.[24-25]

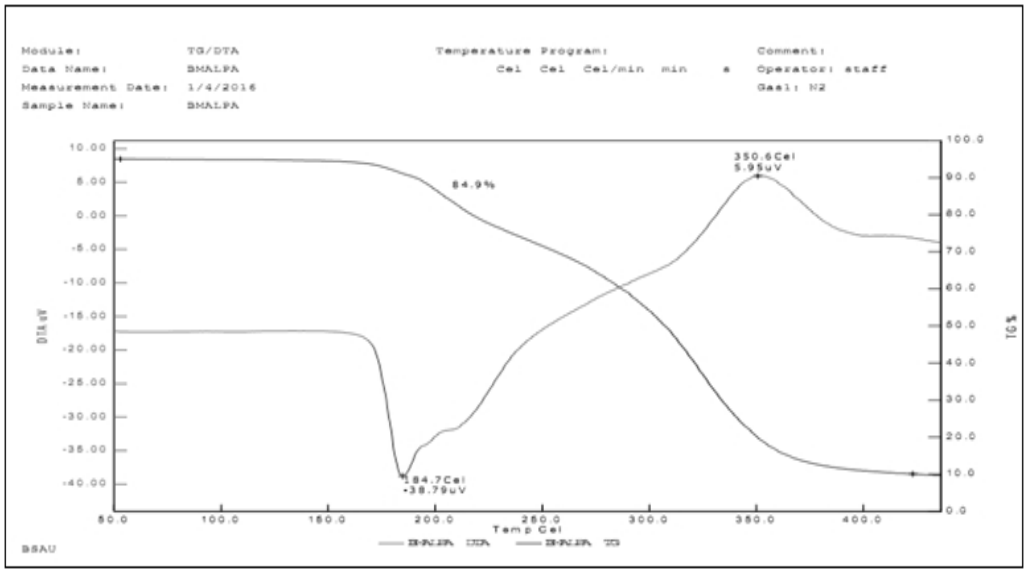

Figure 6
TG-DTA curve of BMALPA

The efficient concentration or minimum inhibitory concentration value for the title compound in microgram is found to be 140.47. The title compound BMALPA possess higher antioxidant activity and higher antiradical power (1/IC $_{50}$) 0.00712, indicates that it can act as a good antioxidant material. The sample is diluted in DMSO to the optimum concentration and the CV graph obtained is shown in (Fig 7), indicating the electrochemical behavior of the title compound.[26-29]

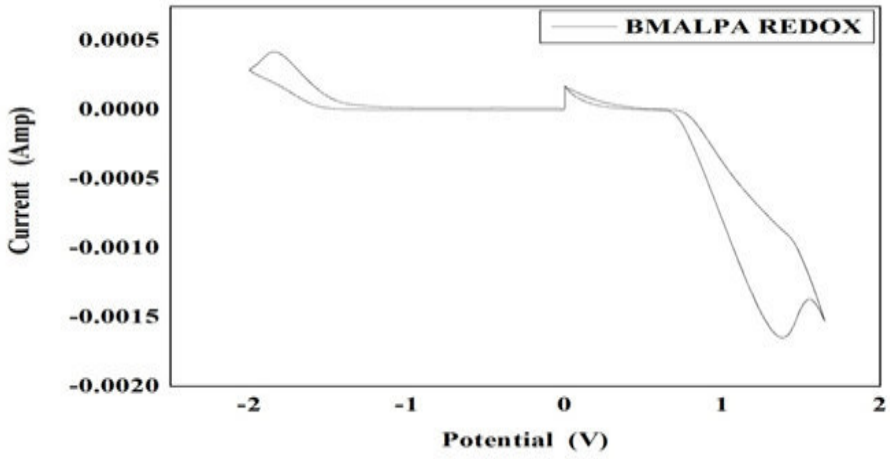

Figure 7
CV of BMALPA

The title compound is showing the suitability of acting as an anticancer material since it is showing high cytotoxicity and low cell viability for liver carcinoma cell line and less toxicity for normal Vero cell line as shown in Table 2 and Table 3. Positive control (PC) used for both the measurement is Cyclophosphamide. [30-31] The IC_{50} value of the title compound is found to be 58.08 µg and 132.33 µg respectively for HEP G2 cell - line and Vero cell - line.

Table 2
Percentage of cell viability and cytotoxicity of Sample BMALPA against HepG-2 cell line

Test	Sample - BMALPA (µg)					
	25	50	75	100	125	PC
% of Viability	53.69	50.33	48.66	45.05	43.27	40.89
% of Cytotoxicity	46.31	49.67	51.34	54.95	56.73	59.11

Table 3
Percentage of cytotoxicity of Sample BMALPA against VERO cell line

Test	Sample - BMALPA (µg)					
	25	50	75	100	125	PC
% of Viability	73.47	64.11	60.72	57.67	51.52	40.89
% of Cytotoxicity	26.54	35.89	39.28	42.23	48.48	59.11

The title compound was subjected to agar well diffusion method for antibacterial and antifungal activity and both were found to have no activity compared to that of their respective positive control. [32-35]

CONCLUSION

The high antioxidant activity, high antiradical power and the in vitro anticancer activity study using liver carcinoma HepG2 cell line and Vero cell line of the synthesised title compound having more hydroxyl groups indicate the possilbilty of utilising the title compound for treating deadly cancerous diseases by scavenging the ROS generated during metabolic processes. The termination of chain reaction by scavenging ROS, the electrochemical behaviour of the title compound confirms its suitability for the anticancer application.

CONFLICT OF INTEREST

Conflict of interest declared none.

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