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RESEARCH ARTICLE

Synthesis, Structural Characterization, Catalytic, Biological and α-Glucosidase Inhibitory Studies of Metal Complexes with Flavone Derivatives

R.R. Krishna Jyothi[1] • Sharow Geeth Vincent[2] • J. Joseph[3*]

[1]*Department of Chemistry, Noorul Islam Centre for Higher Education, Kumaracoil, Tamil Nadu, India.*

[2]*Department of Chemistry, Noorul Islam Centre for Higher Education, Kumaracoil, Tamil Nadu, India.*

[3*]*Department of Chemistry, Noorul Islam Centre for Higher Education, Kumaracoil, Tamil Nadu, India.*

E-mail: josephniche@gmail.com

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ABSTRACT

Heterocyclic compounds, in particular oxygen-containing heterocyclic compounds, are of special interest to medicinal chemists because of their unusual biological properties. In the present study, the highly conjugated nitrogen heterocyclic scaffold comprised of flavone derivative with metal acetates to form metal chelates of the type $[M^{II}L(OAc)_2]$, flavone analogues (L); M=Co^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+}. The above title compounds were characterized using composition analysis of CHN and spectroscopic techniques. Based on spectroscopic and analytical measurements confirmed that square planar arrangements for the Co^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} complexes. Antimicrobial efficacy of prepared complexes were assessed against *A.flavus*, *A.niger*, *B.subtilis*, *E. coli*, *C. albicans* and *S.aureus*. The anti-mycobacterial ($H_{37}Rv$) efficacy of flavone analogues and its complexes were screened using MABA approach and compared with standard. The acetylcholinesterase (AChE) inhibitory effect of the ligand was examined to find out the therapeutic efficiency of compound in the treatment of neurodegenerative disorders. The synthesized ligand exhibited selective inhibition (AChE & BuChe) values (IC_{50} : 0.20 (flavone analogue), 2.41 (Rivastigmine) and 3.01 μM (Galantamine), respectively. Further, the in vitro anti-inflammatory efficiency of metal chelates were performed with the help of egg albumin method. The α-glucosidase inhibition activity was also carried out for the prepared metal complexes.

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Introduction

The emergence of multidrug resistant pathogens exposed the emerging need for novel antimicrobial agents with multi target interactions. The acetylcholine (neurotransmitter) is essential for the treatment of memory and learning in neurodegenerative disease, and the lower level of patients with Alzheimer's disease (AD).

Acetylcholinesterase (AChE) as a neurotransmitter which modulates the acetylcholine and other esters of choline and terminates essential brain functions [Cheung J, Rudolph MJ, Burshteyn F, 2012]. It plays an imperative action in the formation of fibrils through the aggregation of amyloids [Sonmez F, Zengin Kurt B, Gazioglu I, 2017; Tripathi RKP, M Sasi V, Gupta SK, 2018; Soyer Z, Uysal S, Parlar S, 2017; Ali AE, Elasala GS and Ibrahim RS, 2019]. The primary therapeutic strategies for anti-AD to reduce the speed of denaturation of

* Corresponding author: josephniche@gmail.com

acetylcholine (ACh) with the use of acetylcholinesterase inhibitors (AChEIs) [Giacobini E,, 2000; Digiacomo M, Chen Z, Wang S, 2015; Kosak U, Brus B, Knez D, 2016]. As of 2019, around 70 million people worldwide with AD and with an exponential way to reach 140 million by 2030 and so on may be increasing [Ahmad K, Naseem HA, Parveen S, Shah H and Ashfaq M, 2019; Venkatachalam TK, Bernhardt PV, Noble CJ, Fletcher N and Reutens DC, 2016; Abdel-Rahman LH, Abu-Dief AM, Ismael M, Mohamed MAA and Hashem NA 2016]. Numerous studies have documented that metal chelators have beneficial effects in treatment with AD. Evidence from the literature pointed out that 1,10-phenanthroline and 8-hydroxyquinoline derivatives, both of which are lipophilic in nature, have been shown to counteract the effect of A-toxicity by inhibiting its accumulation in infected cells by metal chelation in AD mice. Researchers on the pharmacological studies and the development of N-heterocyclic derivatives (1,10-Phenanthroline derivatives) as chemotherapeutics have developed a lot of interest in over the past few decades to address these factors and have become a promising area of study. N-Heterocyclic analogues are undergoing chelation with the metal ion which make the binding of DNA and greater ability to bind double strands to DNA via different interaction modes [Sathiyaraj S, Sampath K, Butcher RJ, Pallepoguc R and Jayabalakrishnan C, 2013; Nair MS, Arish D and Johnson J, 2016; Chu YC, Wang TT, Wang LJ, Luo QY and Zhu HL, 2019; Daravath S, Vamsikrishna N, Ganji N, Venkateswarlu K and Shivaraj, 2018; Venugopal N, Krishnamurthy G, Bhojyanaik HS and Krishna PM, 2019].

Generally, metal ions play central roles in the structural organization of microorganism and in the biological functions of various enzymes. Literature evidences highlighted that metal complexes showed significant achievements in therapeutic efficiency. The utilization of metals/metal ion in drug architecture has expanded attention because of the cisplatin [Kareem A, Khan MS, Nami SAA, Bhat SA and Nishat N, 2018; Hernández-Ayala LF, Flores-Álamo M, Escalante-Tovar S, Galindo-Murillo R and Ruiz-Azuara L, 2018]. Structural modification of organic ligands with metal complexes is used in the treatment of malignant diseases as chemotherapeutic agents including many forms of cancers [Mandal S, Das M, Das P, Samanta A and Saha NC, 2019; Iftikhar B, Javed K, Khan MSU, Akhter Z and Mckee V, 2018]. Inspired by these literature results, the ligand preparation was attempted through the condensation of flavone derivative and phenylenediamine. We underwent complex formation with the ions Co^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} complexes (Scheme 1). With the aid of analytical and spectroscopic techniques to characterize the ligand and metal complexes. Further, the *in vitro* anti-inflammatory efficiency of metal complexes was performed using egg albumin method. The α-glucosidase inhibition activity was also carried out for the prepared metal complexes.

Experimental: Materials and Methods

All chemicals were procured from sigma and Himedia. TLC tracked the path of the chemical cycle with the aid of pretreated, silica gel-coated plates. Using column chromatography approach, the proper choice mesh and size (60–120 size) of Silica gel was implemented for separation and purity purpose. The chemical composition like CHN analysis were recorded with the assistance of Carlo Erba EA1108 analyzer. The proportions of metal content were measured using gravimetric approach; copper, nickel, cobalt and zinc as cuprous thiocyanate, nickel dimethylglyoximate, cobalt pyridine thiocyanate and zinc ammonium phosphate, respectively. The FT IR spectra was recorded using Shimadzu FTIR Affinity-1 Spectrophotometer. The electronic absorption spectra were recorded using Systronics UV-Visible spectrophotometer (200-1000 nm region). The nature proton and anionic coordination sites were ascertained using proton NMR spectrum with the help of BRUKER 400 MHz spectrometer. The FAB mass were recorded (JEOL SX 102/DA-6000 mass spectrometer/data syste) and predicted the molecular mass & fragmentation of mode of ligand and metal chelates using. The systronics conductivity bridge was utilized to measure the molar conductance and magnetic moment also calculated using Guoy's electronic balance. The electrochemical features of metal chelate were examined using CHI 604D ($n\text{-}Bu_4NClO_4$ electrolyte). The thermal analysis of complexes was performed (Perkin Elmer instrument) under nitrogen atmosphere.

Synthesis of Ligand

The nitro substituted flavone (20 mM) was taken in 50 mL ethanol and 10 mM ethanolic *o*-phenylene diammine was added drop wise in the RB flask. The reaction admixture was heated with stirring for 4 hrs. The reaction movement was monitored using TLC. The observed solid precipitate was filtered and separated out using filtration. The product was dried using vacuum desiccator. Flavone derivative(L): Formula: $C_{36}H_{22}N_4O_6$, Molecular mass 606. Yield: 72%; elemental composition: Calcd for; C 71.28, H 3.66, N 9.24; Found: C 71.08, H 3.52, N 9.15. UV (nm): 340, 250. FT-IR (cm^{-1}): 3090-3070 (Aromatic–H); 1658 (>C=N). ^{1}H-NMR (ppm): 4.8 (-HC=C<, flavone scaffold, 2H, singlet), 6.9-7.8 (20H, multiplet). Mass: molecular ion (found) m/z 607.

Synthesis of Complexes with Ligand

Equimolar hot ethanolic solution of flavone derivative and metal acetate(s) (0.05 M) in 50 mL was taken in round bottom flask. The admixture of reaction solutions was thoroughly stirred and refluxed for 5 hrs. Then, the solid precipitate was formed and separated with aid of repeated washing (Petroleum ether). The other complexes were prepared using the above procedure.

Cu^{2+} chelate: Formula: $C_{40}H_{28}N_4O_{10}Cu$. Molecular mass.788. Yield (%): 65; elemental analysis: Calcd for: C 70.21, H 4.71, N 2.97, Cu 6.75; Found: C 70.07, H 4.63, N 2.77, Cu 6.45. UV (nm): 375, 256 & 490. FT-IR (KBr disc): 1636 υ(>C=N), 540 υ(M–N), 482 υ(M–O). Molecular ion (mass): *m/z*: 789 [M+1]. μ_{eff}(BM) = 1.90. Λ_m (ohm^{-1} cm^2mol^{-1}) =12. Ni^{2+} complex: Formula: $C_{40}H_{28}N_4O_{10}Ni$. Molecular mass. 784. Yield(%): 60; elemental analysis: Calcd for; Carbon 72.58, Hydrogen 3.75, Nitrogen 2.94, Nickel 7.28; Found: Carbon 72.44, Hydrogen 3.60, Nitrogen 2.80, Nickel 7.18. UV-Vis., (nm): 338, 250, 680, 758 nm. FT-IR (cm^{-1}): 1640 υ(C=N), 540 υ(M–N), 488 υ(M–O). molecular ion (Mass): *m/z*: 785 [M+1]. μ_{eff}(BM) = 0. Λ_m (ohm^{-1} cm^2mol^{-1}) = 15. Co^{2+} complex: Formula: $C_{40}H_{28}N_4O_{10}Co$. Molecular mass. 785. Yield(%): 66; elemental analysis: Calcd for: Carbon 72.50, Hydrogen 3.92, Nitrogen 2.94, Cobalt 7.30. Found: Carbon 72.34, Hydrogen 3.74, Nitrogen 2.82, Cobalt 7.20. UV-Vis., (nm): 342, 240, 560, 780. FT-IR (cm^{-1}): 1620 υ(C=N), 538 υ(M–N), 475 υ(M–O). Molecular ion (mass): *m/z*: 785 [M+1]. μ_{eff}(BM) = 2.68. Λ_m (ohm^{-1} cm^2mol^{-1}) = 10. Zn^{2+} complex: Formula: $C_{40}H_{28}N_4O_{10}Zn$. Molecular mass. 790. Yield(%): 70; elemental analysis: Calcd: Carbon 74.28, Hydrogen 3.81, Nitrogen 2.90, Zinc 7.50; Found: Carbon 70.00, Hydrogen 4.54, Nitrogen 2.86, Zinc 7.42. UV-Vis., (nm): 256, 380 nm. FT-IR (cm^{-1}): 1636 υ(C=N), 558 υ(M-N), 486 υ(M–O). ^{1}H-NMR (ppm): 4.7 (-HC=C<), 2H, s), 6.9-7.8 (20H, m), 1.20 (6H, s, $-CH_3$). Molecular ion (mass): *m/z*: 791 [M+1]. μ_{eff}(BM) = 0. Λ_m (ohm^{-1} cm^2mol^{-1}) = 20.

SOD Activity

SOD activity of prepared metal complexes was measured using NBT assay method and expressed IC_{50} [Jayamani A, Bellam R, Gopu G, Ojwach SO and Sengottuvelan N, 2018].

Antituberculosis Activity

Antituberculosis activity of title complexes were recorded with the aid of Microplate Alamar Blue Assay (MABA) [Jayamani A, Bellam R, Gopu G, Ojwach SO and Sengottuvelan N, 2018].

Antimicrobial Activities

The efficiency of title chelates in vitro antimicrobials were screened against microbial species [Jayamani A, Bellam R, Gopu G, Ojwach SO and Sengottuvelan N, 2018]. The selected microorganisms were progressed at optimum temperature in suspended liquid broth for 24 hrs. The bacterial progression was tested after 18 hrs by measuring the turbidity. The MIC was calculated and summarized in the table 1.

a -Glucosidase Inhibitory Assay

The α-glucosidase inhibitory efficiency was performed using modified methodology of Pistia Brueggeman and Hollingsworth [Benhassine A, Boulebd H, Anak B, Ali MK and Belfaitah A, 2019]. Here, the prepared metal complexes were prepared at various concentrations (20 to 400µg/mL), 100µL phosphate buffer and 10 µL enzyme were mixed thoroughly and incubated at RT for 20min. The α-glucosidase inhibitory response was started after the incubation time with a slow addition of 10 µL pNPG (substrate) and incubated again for 30 min. 50µL Na_2CO_3 (0.02 M) was added drop-wise to terminate the reaction and noticed at 405 nm. The IC_{50} values of prepared complexes were calculated and tabulated.

Anti-inflammatory Studies (Egg Albumin Denaturation Technique)

The prepared flavone analogues and its metal complexes compared with diclofenac at various amounts of 10µM, 50µM, 100µM, 250µM and 500µM mixed in double distilled water and phosphate buffer. The reaction admixture 2 mL and 1% egg albumin solution of 1 mL in buffer was kept at 37 °C for 20 min at incubator. The denaturation of egg albumin was convinced in the admixture at 65°C in water bath for 15 min. The prepared chelates were assessed for anti-inflammatory efficiency with the help of denaturation of egg albumin protocol. In this assay, the denaturation induces the change of absorbance at 660 nm were noticed with the help of UV-Vis double beam spectrophotometer. The diclofenac was utilized as standard. The observed outcomes were compared with standard drug / literature resources at different concentrations (10, 50, 100, 250, 500µM/mL). The inhibition percentage (IC_{50}) was arrived using the formula as follows:

% denaturation of egg albumin inhibition = $[(A_t-A_c)/A_t] \times 100$

where A_t and A_c are absorbance of test and control respectively.

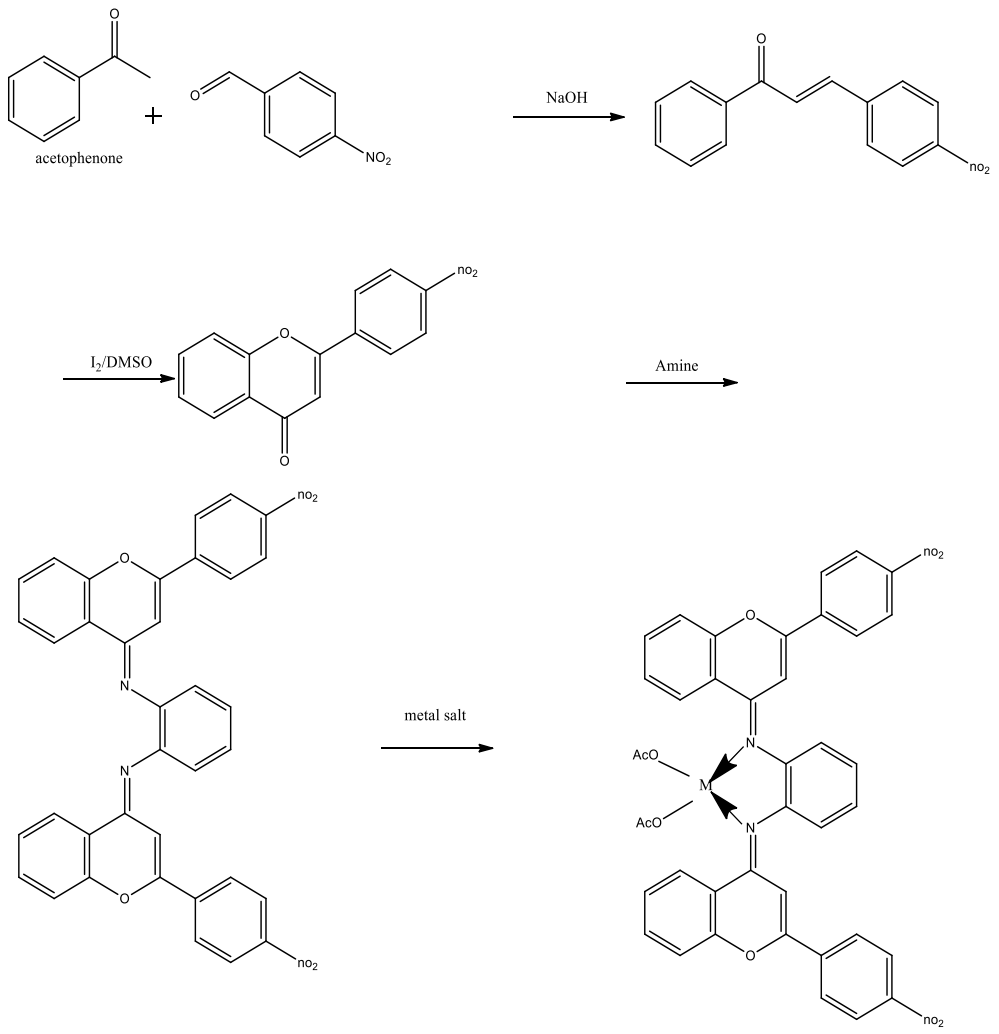
AChE/BChE Inhibition Studies

Ellman's spectrophotometric technique has determined the inhibitory potency of the novel flavone derivative on BChE / AChE activities. As substrates for both cholinergic enzymes (AChE / BChE), butrylcholine iodide (BChI) / acetylthiocholineiodide (AChI) is used. In short, the solution mixture of 50 µL of Tris / HCl buffer and varying concentrations of samples (25-100 µL), 50 µL of BChE / AChE solutions (5.32 as well as 10^{-3}EU solutions). Then, the reaction mixture was incubated at 30 ° C for 10 min. Further, 50 µL of DTNB (0.5 mM and 25 mL) was added to the incubated mix and analyzed at a wavelength of 412 nm using spectrophotometric analysis.

Results and Discussion

Chemistry

Material elucidation is the significant analysis to arrive the structural and physico chemical features of synthesized compounds/ metal complexes. The present study was originated based on the outcomes of chemically modified flavone derivatives as excellent structural core for both optical and pharmacological utilities. In the present study was envisioned on the suitable structural modifications and biochemical investigation make the title compounds as emergent materials for catalytic and medicinal applications. The characterization was achieved using different analytical and spectral techniques which includes solubility and stability of materials (inferred from thermal behaviour). The synthesis of desired flavone analogues was performed from flavone molecule. In the first step, the reaction of substituted aromatic aldehyde with acetophenone leads to the formation of flavone and followed by the reaction of 4-methoxy-o-phenylenediammine to arrive flavone derivative. Furthermore, the desired metal complexes were formed by the reaction of flavone analogues and respective metal acetates. The product formation was confirmed by the observation of single spot noticed in the TLC. The prepared compounds and complexes were stable at room temperature (as evidenced from TG measurements). The metal chelates are coloured, crystalline and exhibited different colours (based on the structural conjugation). They showed solubility in the organic solvents DMSO. Numerous attempts were taken to prepare crystals for the title complexes and achieve structural features. The molar conductivities (10-20 mho $mol^{-1}cm^2$) of 10^{-3} mol L^{-1} in DMSO solution of metal complexes with flavone derivative indicate their non-electrolytic behaviour [Abdel-Rahman LH, Abu-Dief AM, Ismael M, Mohamed MAA and Hashem NA, 2016]. It was confirmed that the anion participates in coordination with metal ion (confirmed by FT IR & TGA).



Where
M = Cu(II), Co(II), Ni(II) & Zn(II)
Scheme 1 Schematic representation of synthesis of title complexes
Fig. 1.

IR Study

The vibrational frequencies of flavone analogue and metal complexes were noticed using FT IR technique. The pictorial representation of ligand vibrational frequencies (Fig.1), the imine (>C=N) stretching vibration at 1640 cm^{-1} verified by the ligand formation (condensation of flavone derivative with 4-methoxy-o-phenylene diamine). There is a shift in the above vibrational frequency (20-36 cm^{-1}) indicates the coordination of imine scaffold 's nitrogen donor atom transfers lone pairs of electrons to metal ions with coordinate bonds (Fig.2). Furthermore, the new vibrational frequencies was noticed at 430-458 cm^{-1} and 492-530 cm^{-1} region corresponds to υ(M-O) and υ(M-N), respectively [Jayamani A, Bellam R, Gopu G, Ojwach SO and Sengottuvelan N, 2018]. In addition, the nature coordination of acetate ion to metal was confirmed based on the frequencies, 1550-1560 cm^{-1} and 1310-1368 cm^{-1} indicates the monodenate chelation of acetateion.

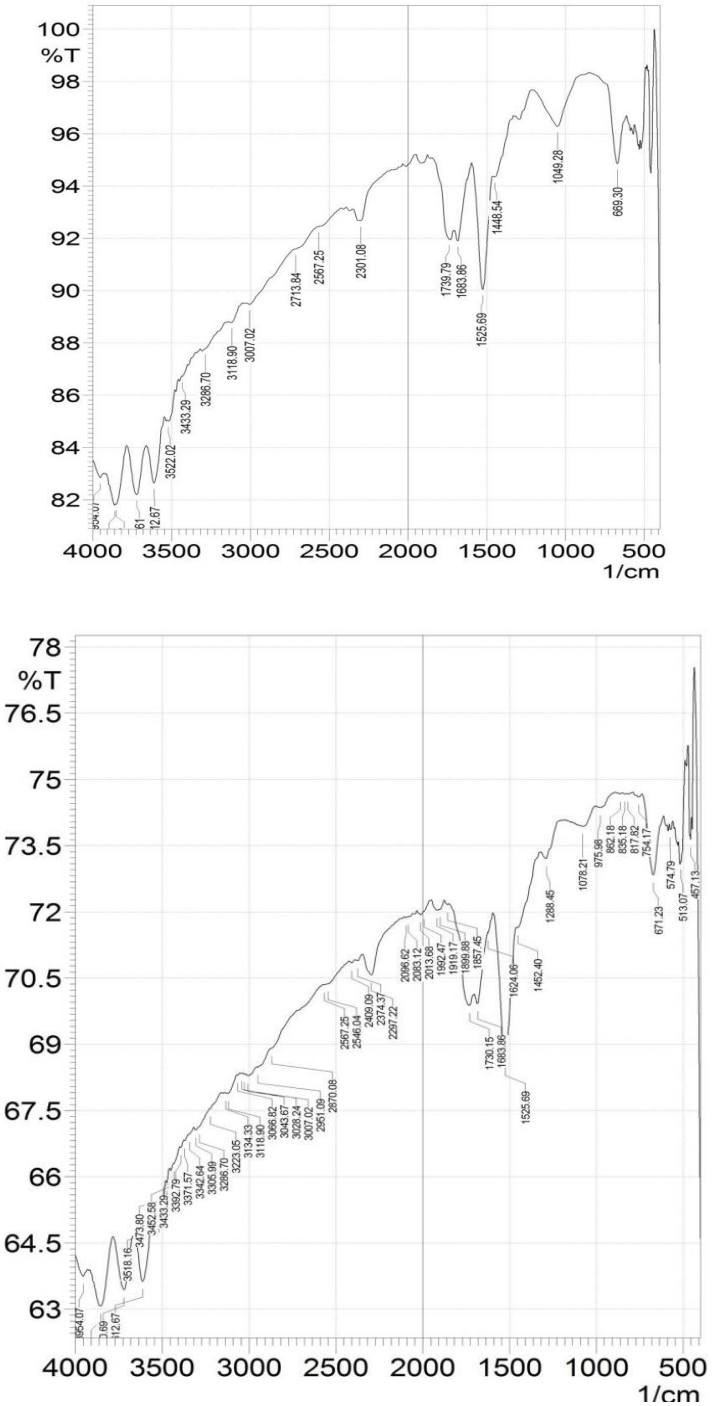


Fig.2. IR spectrum of ligand (a) and copper complex (b)

NMR Measurements

The H-NMR spectrum of flavone derivative was measured in $CDCl_3$ and TMS as standard. The ligand spectrum (fig. 3), the appeared peak at 6.9-7.8 ppm which corresponds to aromatic C-H protons. Further, there is a peak was seen at 4.8 ppm which corresponds to CH=C< proton in the flavone moiety and moved to downfield region in the case of zinc chelate spectrum (fig. 4). This observation authenticated that >C=N coordinate with Zn^{2+} ion. Further, the new peak was seen in the zinc complex at 1.20 ppm which corresponds to $-CH_3$ protons (acetate ion). Therefore, these observed peaks and assignments were recognized with the structural arrangements of flavone analogue and its zinc chelate as outlined (Scheme 1). The ^{13}C-NMR spectrum of flavone derivative showed the peaks were agreement with the different nature of carbon atoms as compared with related compounds in the literature sources (fig. 5).

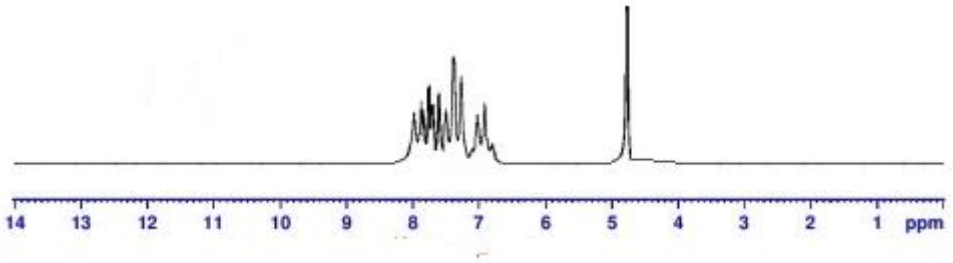


Fig. 3. ^{1}H-NMR spectrum of ligand

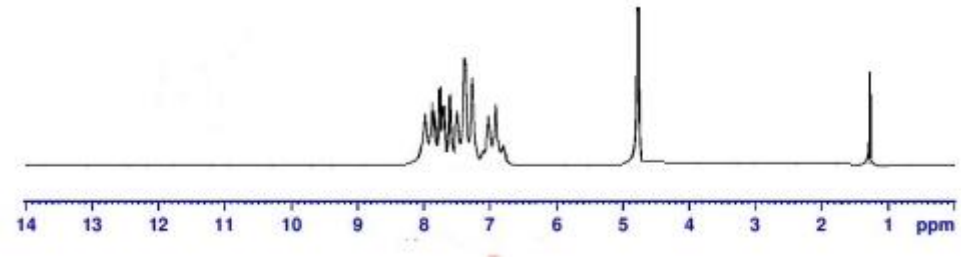


Fig. 4. ^{1}H-NMR spectrum of zinc complex

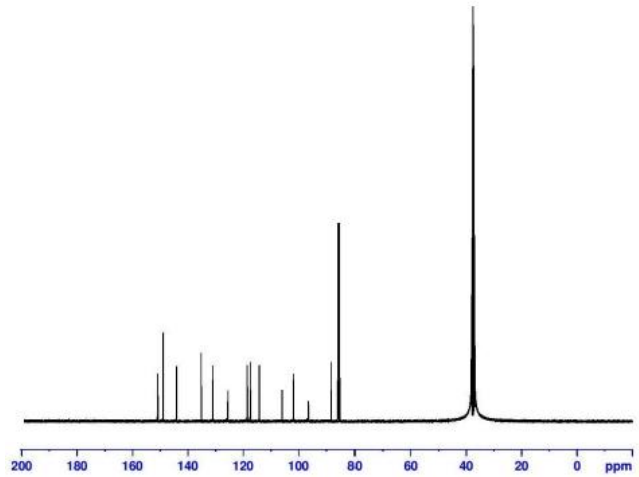


Fig. 5. ^{13}C-NMR spectrum of ligand

FAB Mass Spectral Studies

Molecular mass (m/z) values gave some confirmation of structural formulae of flavone analogue and metal chelates. The ligand mass spectrum conforms with the suggested formula (molecular ion m/z 607, Fig.6). The molecular mass peak in the copper complex was perceived at m/z 789 (Fig.7) which authenticates that metal complex is monomeric in nature. Additionally, the mass spectra show various peaks reflecting successive degradation of the complexes with an intensity gives an indication of fragment stability. Therefore, it was developed that the molecular mass and conductivity values of metal complex stoichiometry as $[ML(OAc)_2]$.

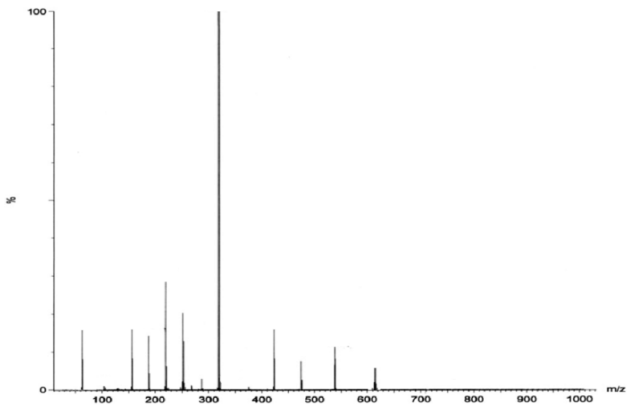


Fig. 6. FAB Mass spectrum of ligand

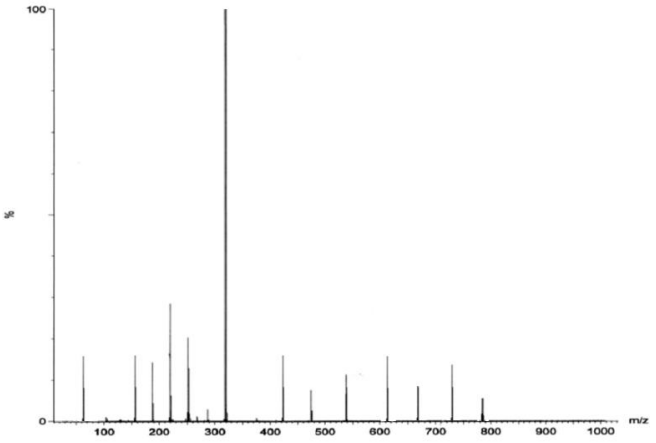


Fig. 7. FAB Mass spectrum of copper complex

Stability of the Complex

The solubility and stability of metal complexes were measured in DMSO solvent. It was utilized in the biological screening and other measurements. To find out the stability of complex upon dissolution in DMSO solvent, its rate of decomposition was monitored using absorption spectrum. In the case of cu^{2+} complex, no noticeable changes in the UV-Vis spectrum with an interval of 1 hr (data not shown). The molar conductivity measurement was also recorded for the above complex with different time interval. There are no significant changes in the conductance values (20 $\Omega^{-1}cm^2mol^{-1}$) of complex demonstrated the non-electrolytic behaviour of complex and maintain its structure in solution during the different time interval. Therefore, based on these experimental observations stated that the complexes are stable and may be utilized in the biological studies.

Thermo Gravimetric Analysis

The thermogravimetric profile of metal complexes were recorded from 0 to 1000°C under nitrogen atm and heating rate 10°C/min. In the case of copper complex, no decomposition was seen upto 200°C exposed that no water molecules in the complex stoichiometry. During the temperature range 250-280°C, there is a slight deviation from the straight line indicates the decomposition of acetate ion and followed by ligand structural core partially. Further, the ligand decomposition was observed in the in the temp range 300-440°C. Finally, the solid residue (CuO) was seen in the spectrum along with small amount of ash. Similar thermogravimetric features was also observed for the other metal complexes. Thermal stability is a noteworthy feature in optoelectronic applications (material without any decomposition at high temperature is essential for OLEDs). Thermogravimetric analysis was adopted to predict temperature withstand capacity of prepared complexes. Materials with high T_i values are essential factor that decides suitability for device fabrications with improved performance. Therefore, the above observation concluded that the different decomposition steps was in accordance with respect to temperature and excellent arrangement with the structural formulae of the metal chelates as indicated in the scheme 1.

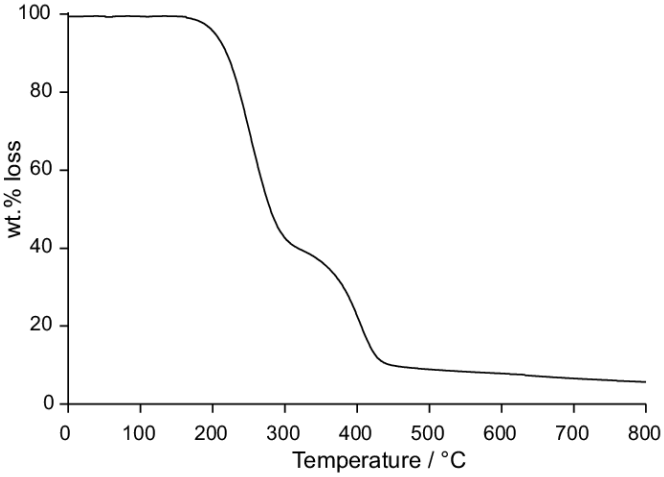


Fig. 8. TGA profile of copper complex

Electronic Absorption Spectra

Ligand electronic absorption spectrum showed two peaks at 250 & 340 nm which are recognized as transitions to π-π * and n- π *. These transitions have been moved to lower wavelength in the spectra of metal complexes, and some important shifts are correlated with donor atoms involved in the bonding between metal and ligand. The electronic absorption spectrum of Co(II) complex exhibited two bands in the region 560 and 780 nm corresponds to $^4T_{1g}\rightarrow{}^4T_{2g}(F)$, $^4T_{1g}\rightarrow{}^4A_{2g}(F)$ transitions. Its magnetic moment value of 2.68 B.M suggested for square planar geometry of Co(II) complex [Manimohan M, Pugalmani S and Sithique MA, 2019]. The electronic spectra of copper(II) complex was exhibited a broad band at 490 nm which may be assigned to the transition $^2B_{1g}\rightarrow{}^2A_{1g}$. Based on the absorption bands, an square planar geometry was assigned for Cu(II)complex [Bougherra H, Berradj O, Adkhis A and Amrouche T, 2018]. The magnetic value of 1.92 B.M. which further supports the square planar geometry of Cu(II) complex. The UV-Vis., spectra of Ni(II) complex showed charge transfer transition in the absorption region 758 & 680 nm. The observed transition shows $^3A_{2g}(F)\rightarrow{}^3T_{2g}$ (F) and $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$, were dependable with well-defined square planar configuration around ligand structural core. The nickel complex also exhibited a distorted square planar geometry suggested magnetic value of 0 BM. The synthesized zinc(ll) complexes are diamagnetic compared with totally filled d orbitals (electronic configuration d^{10}).

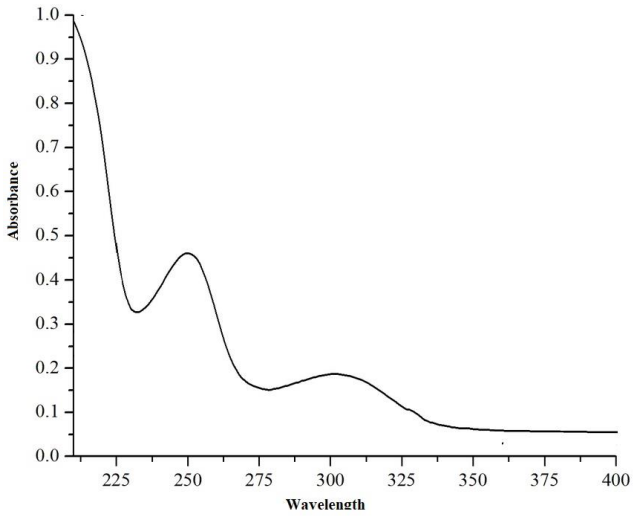


Fig. 9. UV Visible spectrum of ligand

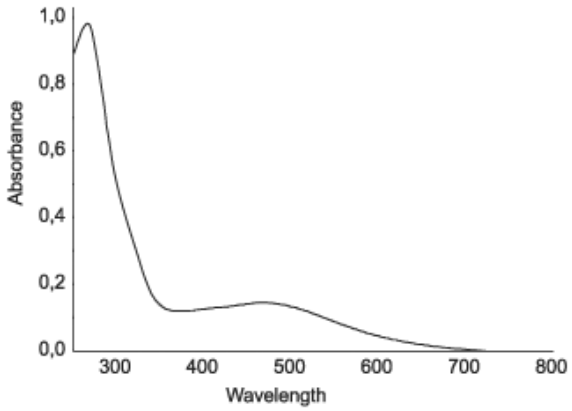


Fig. 10. UV Visible spectrum of copper complex

ESR spectrum

The EPR of Cu(II) complex was measured at RT and LNT. The spectral features were arrived at LNT (showed splitting of lines, g_{II} = 2.2250, $g_{\perp}$ = 2.0632, A_{II}=140 G) which is confirmed the presence of the unpaired electron of d^9 electronic configuration localized in $d_{x^2-y^2}$ orbital for copper(II) ion in square planar geometry [Manjuraj T, Krishnamurthy G, Bodke YD, Naik HSB and Kumar HAS, 2018]. In the present case, the g|| values were obtained for copper complex 2.260 which indicated that the covalent character of the ligand-metal bond. In addition, 151 (gII / AII) was found to be the f value for the prepared Copper complex. The value for Cu,Zn SOD of the geometric distortion factor 'f' is 160 cm, suggesting a distortion from normal geometry, and is important for catalytic and pharmacological activities. Therefore, the prepared copper complex exhibited appreciable distortion from regular geometry shape and improved SOD and catalytic activities as compared with known standard and reported compounds (similar molecular architecture). The α^2 for the copper complex was also calculated. In the present case, the value of α^2 (0.75) suggesting that the complex possessed mixing of ionic and covalent character.

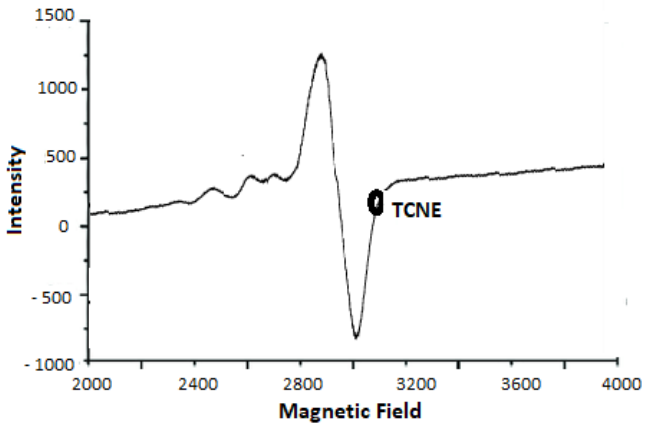


Fig.11. ESR spectrum of copper complex at 77 K

Powder X-ray Diffraction

P-XRD analysis of prepared title complexes was performed to arrive more structural and particle nature of synthesized compounds/ complexes. PXRD was recorded at ambient temp in the range 2θ = 10° - 80° and its diffraction patterns summarized. In the case of flavone derivative, there are three sharp peaks was observed with a 2θ values, 15.30°, 20.40° and 38.50° corresponding to interplanar spacing (relative intensity) 5.40 (52.75%), 4.20 (75.05%) and 2.68 (46.10%), respectively. In the case of copper complex, one peak was vanished as compared to ligand and arrival of new peaks signifying the formation of metal complexes with crystalline nature. Furthermore, the powder diffraction pattern of Cu(II), Zn(II) and Ni(II) chelates revealed peaks specifying crystalline features whereas Co^{2+} complex showed its amorphous nature.

Antimicrobial Activity

The prepared flavone analogue and its metal complexes were screened for their inhibitory efficiency on the growth of different bacterial (*Aspergillus flavus, Escheria coli, Candida albicans, Staphylococcus aureus, Aspergillus niger* and *Bacillus subtilis*. The experimental observations were summarized in table 1 and demonstrated that the metal chelates are more powerful in preventing the growth of microbial species as compared to flavone derivative under similar experimental conditions. The observed trends are may be due to chelation. In addition, chelation reduces the metal ion 's polarity mainly due to sharing of its positive charge with the donor atoms within the whole ring system. The experimental results indicated better activity of the prepared compounds towards fungi than bacterial species due to the difference in the composition of the cell membrane.

Table 1. MIC values of ligand and its metal complexes against different microbial species (µg/mL)

Compound/ standard	*E. coli*	*S. aureus*	*B. subtilis*	*C.albicans*	*A.niger*	*A.flavus*
Ligand, L	88	90	84	80	98	94
[CuL(OAc)$_2$]	36	30	24	26	20	28
[NiL(OAc)$_2$]	48	40	36	32	38	42
[CoL(OAc)$_2$]	46	42	40	34	38	44
[ZnL(OAc)$_2$]	40	34	30	32	28	32
Sreptomycin	8	12	6	14	10	15

In the mechanistic aspects, the metal chelates diffuse into the specific target site (through the lipid layer of cell membrane) and destroy them by combining some cell enzyme groups with imine functionality (>C=N-). The complexes of Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} demonstrated superior antimicrobial efficiency against humans. The variation in the efficacy of metal complexes with different microbial species depends on variations in microbial cell ribosome [Ikeda NEA, Novak EM, Maria DA, Velosa AS and Pereira RMS, 2015]. The complexes toxicity mechanism can be attributed to increasing the lipophilic existence of chelates. The mode of response of metal chelates with specific site involves through the disruption progressive of cell wall, cytoplasmic membrane, development of ATP, respiration and oxidative phosphorylation towards the apoptosis of organism.

Anti-tubercular Activity

Anti-tubercular efficiency of metal complexes was performed against M. tuberculosis by MABA method and the results are presented in Table 2. The experimental outcomes highlighted that the metal complexes showed higher anti-tuberculosis efficiency against $H_{37}RV$ strain as compared to the flavone analogue and standard (INH). It is observed that all the metal(II) chelates demonstrated potential inhibitory efficiency as compared to ligand. Among the metal chelates, the Cu^{2+} chelate showed higher activity with MIC value of 5.42 mg/mL due to the incorporation of copper ion into the heterocyclic systems may be responsible for reduced side effects and lower toxicity.

Table 2. Anti-mycobacterial activity results of ligand and its metal complexes

Sl.No	Name of compounds /complexes/ standard	MIC (mg/mL)
1	H_2L	54
2	[CuL(OAc)$_2$]	5.42
3	[NiL(OAc)$_2$]	16.0
4	[CoL(OAc)$_2$]	18.0
5	[ZnL(OAc)$_2$]	10.8
6	Isonizaid	3.2

Lipophilicity Test

Lipophilicity is one of the most significant parameters in medicinal chemistry, and one of the most insightful & active physicochemical properties. In the drug design of chemical molecule depends on lipophilicity determinations [Syed Ali Fathima S, Paulpandiyan R and Nagarajan ER, 2019]. The partition coefficient (log P) indicated the lipophilic nature of metal complexes. The absorption maximum of the metal complexes was determined using UV-Visible double beam spectrophotometer. The λ_{max} for n-octanol is 263 nm. All the observation showed that the complexes have enhanced bioavailability than its corresponding ligands. This liphophilicity tends to increases the efficiency across the lipoidal bacterial membrane due to its highly conjugated system of synthesized metal complexes.

SOD-mimetic Activity of Metal(II) Complexes

In the present investigations that all the metal complexes had excellent SOD-mimetic activity, with IC_{50} values ranging from 0.80 to 1.10 µM (the smaller the value, the greater the SOD-mimetic activity). Owing to its presence of flavone structural core, electro with drawing nitro substituent and metal ion makes complexes showed greater SOD activity. The higher biological activities of copper complexes may be attributed to the flexible ligands, which are able to accommodate the geometrical change from Cu^{II} to Cu^{I}, It has been suggested that electron transfers occur through direct binding between copper(II) and superoxide anion radicals; The copper complexes therefore displayed greater SOD activity than other metal complexes. This observation has been confirmed by geometry distortion (the "f" factor value). The synthesized copper complexes have a greater geometry distortion.

Antioxidant

The metal chelates of flavone ligand were performed for their screening of antioxidant potency with the help of DPPH radical scavenging assay method. The experimental outcomes were indicated that metal complexes have shown the antioxidant efficacy. Among the metal chelates, the copper chelate is efficient chelate compared with standard, ascorbic acid. Generally speaking, the Hydroxyl radicals are extremely reactive species and proficient of abstracting hydrogen atoms from membrane lipids. The antioxidant activity of flavone scaffold, metal chelates and the standard ascorbic acid were assessed and summarized IC_{50} values. The IC_{50} value of flavone derivative is 80 µg/mL, its metal chelates, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} complexes) 32, 46, 64 and 70 µg/ mL and the standard ascorbic acid (IC_{50} value is 22 µg/mL).

Anti-inflammatory Efficiency

The purchase and utility of animals for pharmacological research work is more complicated process due to some ethical issues. Keep these aspects in mind, in the present work was concentrated on protein denaturation methodology with the help of egg albumin approach to infer out the efficacy of metal chelates. The chemical molecules that may inhibit protein denaturation and thereby improving the anti-inflammatory process. The *in vitro* anti-inflammatory efficacy of metal complexes was assessed against protein denaturation of albumin. Among the prepared metal complexes, copper chelates (IC_{50} 40 µg/mL) displayed higher inhibitory efficiency (IC_{50} 78-90 µg/mL) due to the presence of redox and structural core as compared to Diclofenac (IC_{50} 50 µg/mL). The modulation was seen in the absorbance of metal complexes owing to its inhibition of protein denaturation by metal complexes. The copper complex is active at lower concentration as compared to standard. Hence, the findings of this preliminary analysis can be inferred that coffee possessed an anti-inflammatory effect marked *in vitro* against protein denaturation. Further conclusive studies are required to establish the mechanisms and constituents behind its anti-inflammatory behavior.

α-Glucosidase Inhibition Study

α-Glycosidase inhibitors minimize glucosidase activity in the intestine, slow carbohydrate absorption in intestine, and decrease blood glucose levels. Mainly sugar mimetic compounds are the α-glucosidase inhibitors [Syed Ali Fathima S, Paulpandiyan R and Nagarajan ER, 2019; Medina JJM, Naso LG, Pérez AL, Rizzi A and Williams PAM, 2019]. Long-term utility, however, often yields some unwanted responses causing excessive concealed hazards. Therefore, the above inhibitor must be identified based on the availability and utility with unwanted responses with biological systems. In this regard, the inhibitors of α-glucosidase are a beneficial therapeutic approach for sinking the risk and other associated diseases. In the present study was addressed on evaluation of metal chelates and ligand inhibition efficiency of α-glucosidase. The α-Glucosidase inhibition for copper chelate (0.08 µM) (Table 3) displayed greater inhibitory efficacy than other metal chelates and 1-deoxynojirimycin (standard), α-glucosidase inhibitor, most commonly found in mulberry leaves). The increased complex activity may be due to the electronegative nitro substituents present in the ligand base of the flavone derivative. The efficacy flavone derivative may by modulated by the coordination with soft Lewis acid which makes appropriate variations in the structural or electronic properties and imparts α-glucosidase inhibition. The nature and position of the substituents within the Cu(II) complex modifies the inhibition of α-glucosidase. In this study, the complexes containing nitro substituent may improve α-glucosidase inhibition. Therefore it is proposed that the presence of electronegative nitro group is responsible for the greater efficacy of Cu(II) complex inhibition of α-glucosidase [Zordok WA and Sadeek SA, 2018; Kavitha B, Sravanthi M and Reddy PS, 2019]. Additional *in vivo* studies are therefore necessary to prove their antidiabetic behavior and mechanism.

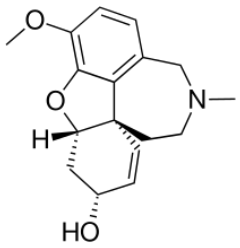
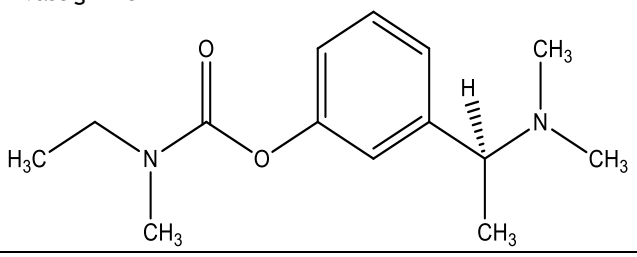
Table 3. α-Glucosidase inhibition activity of metal(II) complexes

Sl.No	Complex/standard	IC_{50} (µM)
1	Copper complex	0.80 ± 0.02
2	Nickel	0.98 ± 0.5
3	Cobalt complex	0.96 ± 0.2
4	Zinc complex	0.84 ± 0.4
5	DNJ	300 ± 0.5

Cholinesterase Inhibitory Activity

Cholinesterase inhibitors (ChEIs) are therapeutic targets that may enhance cholinergic response to boost memory, efficiency, minimize psychiatric and behavioral disorders. The Cholinesterase inhibitory activity (BuChE, AChE) of the prepared flavone scaffold was compared with Galantamine & Rivastigmine as standards with the help of modified Ellman approach and concise in table 4. Some of the inhibitors are Rivastigmine, Donepezil and Galantamine utilized to treat cognitive issues. The experimental observation indicated that the Galantamine (IC_{50} = 2.41 µM), the synthesised highly conjugated flavone derivative with an IC_{50} value of 0.20 µM and Rivastigmine (IC_{50} = 3.01 µM). Based on the comparison of Cholinesterase inhibitory efficacy of title compound with standards expressed as lead molecule with improved potency. Generally, the phenolic compounds interact with residues of amino acids that characterize the active position of AChE through a hydrogen bond, and π-π stacking interaction. Because of greater binding ability, several oxygen functionalities are improved Cholinesterase inhibitory response. This research finding may provide opportunities for researchers to develop new effective inhibitors of ChEs by adequately modulating the pattern of substitution and also some pharmacopores from the viewpoint of multifunctional anti-AD agents. More clinical trials, however, are required to clarify the effectiveness of the flavone derivative in AD management.

Table 4. *In vitro* inhibition IC_{50} values (μM) and selectivity index of compound and standards for AChE and BuChe

Compound Structure	**Inhibitory values IC50 (μM)**		**Selectivity index =IC_{50} (BuChE)/IC_{50} (AChE).**
	AChe	**BuChe**	
Ligand (L)	0.20±0.16	3.2±0.20	16
Galantamine 	2.41±0.10	17.30±0.15	7.21
Rivastigmine 	3.01±0.2	0.30±0.1	0.10

Conclusion

The investigation under way was addressed on synthesis of title chelates with flavone derivative (as per scheme 1). The synthesised compounds/complexes were structurally interpreted with the help of FT-IR, NMR, Mass and CHN. Square planar geometrical arrangements was arrived for the prepared metal complexes, based on analytical and spectroscopic outcomes. The pharmacological efficacy of prepared metal complexes was assessed and compared with standards & related literature reports recommended that the presence of highly conjugated flavone derivatives modulates different biological mechanisms and also mimic natural enzymes. The selectivity index of ligand towards AChE is 16-fold as compared with Galantamine, and Rivastigmine, respectively. The Cu(II) chelate expressed an effective inhibition of α-glucosidase compared to the standard inhibitor (DNJ) and may be significant in future for antidiabetic and other related disorders.

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