doi.org/10.36721/PJPS.2020.33.4.REG.1451-1458.1

Synthesis of 2-nitro aniline derivative of 3, 4, 5-trihydroxybenzoic acid, its metal complexes, characterization and bioassay

Nazish Qurban[1], Khadija Shahid[1], Javeria Riaz[1], Amir Waseem[2] and Rabia Batool[1]
[1]Riphah Institute of Pharmaceutical Sciences, Riphah International University (RIU), 7th Avenue Sector G7/4, Islamabad, Pakistan
[2]Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan

Abstract: Present research work discloses new and novel synthesis of metal complexes (Fe, Cu, Zn, Sb and Sn) of 3, 4, 5-trihydroxybenzoic acid derivative containing aniline moiety with substitution at C-7 position of 3, 4, 5-trihydroxybenzoic acid in order to enhance its biological activities by coupling therapeutic values of transition metals as well. *In vitro* antibacterial and antifungal activities of these compounds has been performed by using agar diffusion method against different bacterial and fungal strains. The free radical scavenging assay was performed by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). Cytotoxic action of compounds was assessed by utilizing the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. The structure of newly synthesized complexes were confirmed by using IR and ^{1}HNMR spectral analysis.

Keywords: Amide derivative of 3, 4, 5-trihydroxybenzoic acid, metal complexes, ^{1}HNMR, anti-bacterial activity, antifungal activity, antioxidant activity, anticancer activity

INTRODUCTION

3, 4, 5-trihydroxybenzoic acid is a renowned organic and phenolic acid found extensively throughout the plant kingdom (Madhavi *et al*., 1995). It is also known as gallic acid. Its contents are rich in tea, gallnuts, sumac, witch hazel (*Hamamelis virginiana*), grapes, berries, mango (in peels and leaves), banana, vinegar and other fruits as well as in the wine and hot chocolate (Rajalakshmi *et al*., 1995). Its molecular weight is 170.12 g/mol with melting point 210ºC. Its molecular formula is C_6H_2 $(OH)_3COOH$. Usually found in two distinctive forms; as a free molecule or in combination with tannins (Verma *et al*., 2013). It is a white, yellowish white or pale-fawn colored crystalline powder (Polewski *et al*., 2002).

It commonly uses in the treatment of psoriasis and external hemorrhoids (Cook *et al*., 1995). In recent research, 3, 4, 5-trihydroxybenzoic acid obtained from grape seeds have been shown to prevent development of amyloid fibrils, one of the prospective origin of Alzheimer's disease and Parkinson's disease (Lu *et al*., 2006). It is cytotoxic against cancerous cells devoid of damaging healthy cells (de Mejia *et al*., 2006). It also possess anti-fungal activity against *Fusarium solani*, *Candida species* such as *Candida albicans*, *Candida glabrata*, *Candida prapsilosis*, C*candida tropicalis* and *Candida krusei (Uozaki et al., 2007)*. Also used as an antioxidant, beneficial for human cells to overcome oxidative damage (Kubola *et al*., 2008). It also has antibacterial and antiviral properties (Buzzini *et al*., 2008). Its synthetic esters (gallates) are renowned for its anti HSV-1 and anti HIV properties (Kratz *et al*., 2008). *In vitro* and *in vivo* studies in human beings, animals and cell culture have showed indication that 3, 4, 5-trihydroxybenzoic acid is frequently accomplish in the pharmaceutical trade for many pharmacological and biological actions (Fazary *et al*., 2008).

Hydrogen/hydroxyl donor group at C-7 position of gallic acid provide best site for derivatization as can be replaced by other functionalities. Therefore, in present research work, 2-nitro aniline derivative of gallic acid is synthesized by replacement of OH group with aniline at C-7 position and further a series of metal complexes using different metals were synthesized from this aniline derivative of gallic acid (ligand). Structure of these compounds and ligand were confirmed by spectral characterization technique i.e. IR and ^{1}HNMR spectroscopy. These compounds were evaluated *in vitro* for antibacterial, antifungal, antioxidant and anticancer activities by using reported procedures (Xia *et al*., 2010).

MATERIALS AND METHODS

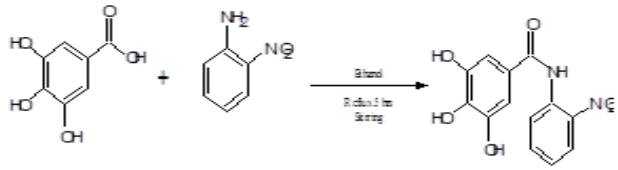
Chemicals utilized as a part of synthesis of ligand and metal buildings were of high review and unadulterated. Organotin halides, 2-nitro aniline and other metal halides utilized in the synthesis were bought from Sigma-Aldrich and were utilized with no further purification. Gallic acid having 97% virtue was transported in from china. Since the reaction was exceptionally sensitive to dampness therefore chemicals utilized for synthesis of metal complexes were dried in situ utilizing standard strategy. Different chemicals like chloroform, methanol, ethanol, acetonitrile, triethylamine were additionally of analytical review.

*Corresponding author: e-mail: nazishsyed.pharmacist@gmail.com

Melting points of synthesized compounds were determined in a fine capillary tube utilizing melting point (Gallenkamp MPA350.BM2.5) mechanical assembly. The infrared spectral analysis was recorded on Schimadzu FT-IR (IR-Prestige-21) spectrophotometer by total reflectance method utilizing ATR 8000A accessory. HNMR spectra were measured on Bruker ARX-300 MHz spectrometer at room temperature using TMS as standard.

Reaction for synthesis of ligand

3, 4, 5-trihydroxybenzoic acid was used as a parent compound and its 2-nitro aniline derivative was prepared. Ligand prepared is usually an amide derivative of 3, 4, 5-trihydroxybenzoic acid or Gallic acid. Synthetic protocol is mentioned in scheme 1.



(1.1) Gallic acid **2-nitroaniline** **(1.2) Ligand**

Scheme 1: Synthesis of Ligand

Synthesis of metal complexes

Metal complexes (Fe, Zn, Cu, Sb and Sn) of 2-nitro aniline derivative of gallic acid (ligand) were synthesized with corresponding metal halides/salts i.e. tin halides (diphenyltin dichloride, trimethyltin chloride, dimethyltin dichloride,), copper (II) acetate monohydrate, antimony triflouride, zinc acetate dehydrate and ferric (III) chloride anhydrous. The synthetic reaction is shown in scheme 2.

Synthesis of amide derivative of gallic acid (ligand) compound 1.2

Gallic acid (1mmol) was suspended in methanol and treat this with 2-nitro aniline (1mmol). The blend was refluxed for 5 hours. The solvent was evacuated through rotary evaporator and the product was recrystallized from ethanol. A light yellow colored crystalline powder was obtained.

Synthesis of metal complexes of ligand

Synthesis of Triorganotin complexes of ligand, compound (1.5)

1mmol of ligand was suspended in dry toluene and treated with triethylamine (1mmol). The blend was refluxed for 3 hours. Triorganotin chloride compound (1mmol) was added in mixture and again was refluxed for 5-6 hours. The dissolvable was expelled through rotating evaporator and the product was recrystallized by using chloroform (Khadija Shahid *et al*., 2009; K Shahid *et al*., 2008; Khadija Shahid *et al*., 2008).

Synthesis of diorganotin complexes of ligand, Compound (1.3), (1.4)

2mmol of ligand was suspended in dry toluene and treated with triethylamine (1mmol). The blend was refluxed for 3 hours. Diorganotin Chloride (1mmol) was added and the mixture was again reflux for 5-6 hours. The solvent was removed through rotary evaporator and the product was recrystallized by using chloroform (Khadija Shahid, *et al*., 2009; K Shahid, *et al*., 2008; Shahzadi *et al*., 2008).

Synthesis of iron (III) complex of ligand, Compound (1.6)

1mmole of ligand and 1mmole of $FeCl_3$ is added in equal volume of ethanol (25ml) separately. Now combine these two solutions with continuous mixing and stirring. Then this mixture was refluxed for 50 minutes. When reaction was complete, resultant shaded solution was left at room temperature. The product was gotten by filtration, washed with ethanol and recrystallized from methanol and dried vacuum desiccator containing silica gel.

Synthesis of copper acetate complex of ligand, Compound (1.7)

Copper complex was synthesized by taking 2mmol of ligand in methanol (solution 1). 2mmol of Cu $(CH_3COOH)_2$. H_2O was added in methanol (solution 2). Solution 1 was mixed with solution 2. Reaction blend was changed in accordance with a PH 7.0±0.5 using triethylamine. The mixture was subjected to stirring for 4 hours at room temperature and precipitates were allowed to settle down by keeping the reaction blend overnight in refrigerator. After consummation of reaction, green shaded precipitates were gotten by filtration and dried over silica gel in vacuum desiccator.

Synthesis of zinc acetate complex of ligand, Compound (1.8)

Ligand (1mmol) and zinc dihydrate (1mmol) were dissolved in equal volume of methanol (25ml) separately, after mixing both solutions pH was adjusted to 7.5±0.5 using potassium hydroxide (0.1% in methanol) and the blend was refluxed for 3 hours. A light yellow product was isolated by filtration and washed with methanol and dried under desiccator containing silica gel.

Synthesis of antimony halide complex of ligand, Compound (1.9)

Complex was set up by mixing solution of ligand (1mmol) in 15ml of acetonitrile and solution of antimony fluoride (1mmol) in 15ml of acetone. The mixture was subjected to mixing for 30 minutes. Arrangement was separated and kept at room temperature for crystallization. The crystallized product got was washed with methanol and dried in desiccator containing silica gel.

Assessment of antibacterial activity

By application of agar well diffusion method, ligand and synthesized metal complexes were screened for anti-

bacterial activity *in vitro* against gram negative and gram positive strains. The agar diffusion assay containing 10ml aliquot of nutrient broth was immunized with living being which is microorganisms and incubated at 37±1ºC for 24 hours. 0.6 ml of nutrient broth of test living being was added to liquid agar cooled at 45ºC blended well and filled sterile Petri dish. Copy plates of every living being were readied. The agar attachments were expelled by hardening agar and required number of openings of 10mm were cut utilizing sterile cork bores. Arrangement of ligand and metal complexes were arranged independently having concentration of 1mg/ml. By utilizing micropipette, 100µl of test was disintegrated in a suitable solvent. Test was filled appropriately marked holes. Antibiotic medication (1mg/ml) solution was utilized as a positive control. Same volume of solvent (as control) and standard antimicrobial specialist antibiotic medication (1mg/ml) were utilized. To permit dispersion of test, plates were set at room temperature for 2 hours and incubated at 37±1ºC for 24 hours. Antibacterial movement was dictated by measuring zone of inhibition in millimeters ((Borges *et al*., 2013).

Assessment of antifungal activity

By using agar tube dilution method, *in-vitro* antifungal activity was performed for ligand and their metal complexes against four (4) fungal strains namely *Aspergillus niger, Candida albicans, Fusarium solani* and *Candida glabrata*. Sabouraud dextrose agar (SDA) was blended by blending 4% Sabouraud glucose agar in refined water with steady mixing and warming. Integrated media was then filled test tubes and autoclaved at 121ºC for 15 minutes. Autoclaved tubes were then permitted to cool up to 50ºC. Arrangement of test tests were arranged independently having concentration of 20µg/ml in sterile DMSO. Nystatin (20µg/ml) arrangement was utilized a positive control. 100µl solution of test and positive control (Nystatin) was moved into various non-solidified sabouraud agar media tubes by utilizing micropipette. At room temperature, tubes were permitted to solidify in inclining position. Every tube was inoculated with 4mm distance across bit of inoculums which were expelled from seven days old culture of parasitic strains. All culture tubes were incubated at ideal temperature of 28-30ºC for 7-10 days. Dampness of incubator was kept up around 40-50%. Cultures were analyzed in any event twice week after week. Following 7-10 days of incubation, test tubes with no unmistakable development of microorganism were taken to speak to the minimum inhibitory concentration (MIC) of test which is expressed in µg/ml (Seo *et al*., 2013).

Assessment of anti-oxidant activity

The free radical scavenging assay was performed by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). Solution was prepared by adding 3.2mg of DPPH in 100ml of chloroform. From this solution (DDPH solution), 2800µl was taken in a glass tube followed by the addition of 200µl of sample solution leading to the final concentration of 62.5 ug/ml, 125ug/ml, 250ug/ml, and 500ug/ml (negative control). These solutions (DPPH and Sample solution) were mixed thoroughly and placed in a dark room for 1 hour. Temperature of room was maintain between 25 to 28^{o}C. DPPH (2800ul) and chloroform (200ul) solution was used as a control. Mixture of methanol 200ul and 82% chloroform (2800ul) was taken as a blank. UV visible spectrophotometer was used to measure absorbance rate at 517nm.The test was repeated 3 times and inhibitory action in percentage was obtained using formula: $[(A_0 - A1)/A_0] \times 100$

Where A_1 indicated absorbance of the samples tested and A_0 represented absorbance of the control (Kulisic *et al*., 2004).

Assessment of anticancer activity against HeLa cell lines

Cytotoxic action of compounds was assessed in 96-well level bottomed smaller scale plates by utilizing the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay (Mosmann, 1983). For this reason, HeLa cells (Cervical Cancer) were refined in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37ºC. Exponentially developing cells were collected, checked with haemocytometer and diluted with a specific medium. Cell culture with the concentration of 6x104 cells/ml was arranged and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was evacuated and 200 µL of crisp medium was included with various convergences of compounds (1-30µM). After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to every well and brooded assist for 4 hrs. Along these lines, 100µL of DMSO was added to every well. The degree of MTT diminishment to formazan inside cells was ascertained by measuring the absorbance at 570 nm, utilizing a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The percent hindrance was figured by utilizing the accompanying equation:

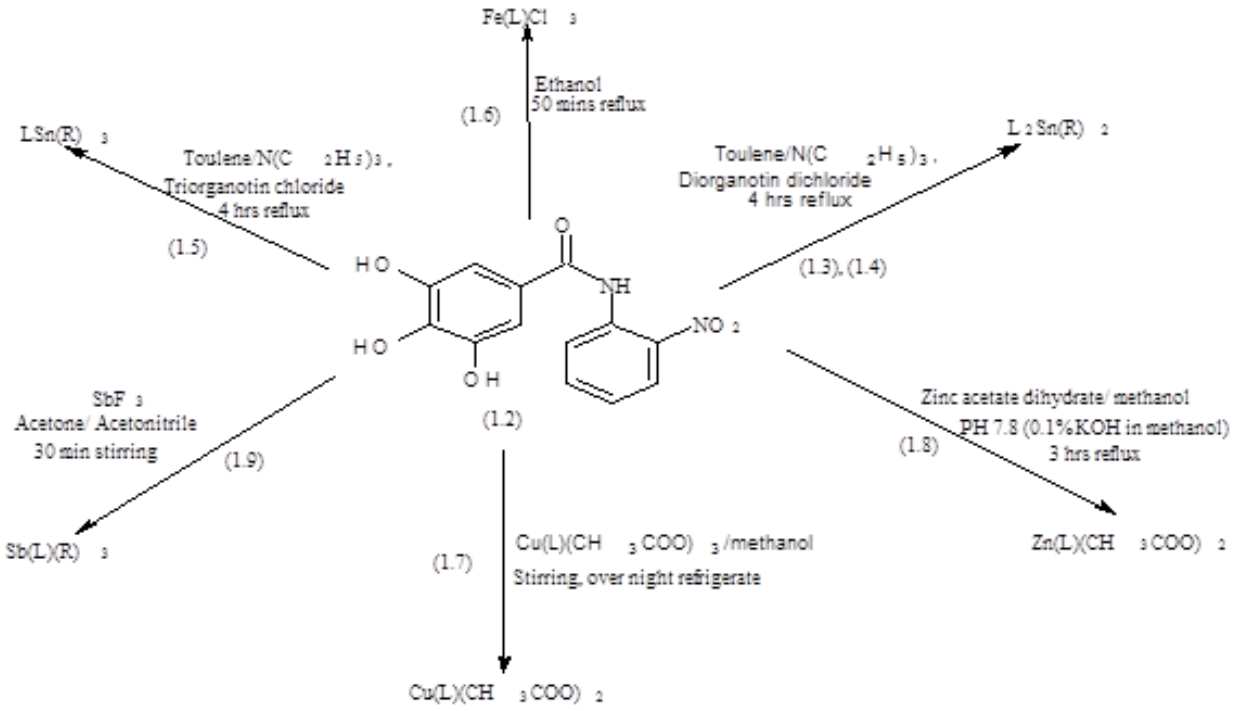
% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control) *100).

The outcomes (% inhibition) were prepared by utilizing Soft-Max Pro programming (Molecular Device, USA).

RESULTS

Spectral data

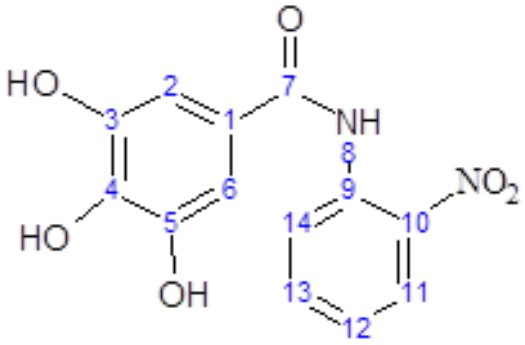
^{1}HNMR spectrum of synthesized compounds were measured on 300 MHz Bruker Avance multinuclear spectrometer. DMSO was used as a solvent. The numbering scheme (3,4,5) for ligand and their metal complexes is shown below:



Scheme 2: Synthesis of metal complexes

Spectral data of ligand (1.2)

IR (cm^{-1}): 3336 (N-H str.), 1697 (Amide C=O), 1489 (C=C), 1581 (N-H bend), 1091 (C-N), 3429 (O-H), 1338 (N-O sym.), 1512 (N-O asym.); ^{1}HNMR (DMSO, 300 MHz) δ 7.244-7.270 (1H each, s, 2,6-CH), 5.87 (1H each, s, 3,4,5-OH), 6.44 (1H, s, CONH), 6.94-7.386 (1H, m, 11, 12, 13, 14-Aromatic H); Yield 89%; m.p. 70ºC; mol.wt. 290.23 (g/mol); yellow crystalline. Numbering of C-atoms in given in scheme 3.



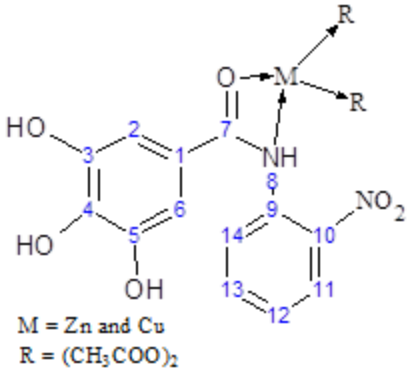
Scheme 3: Numbering scheme for ligand

Spectral data of Diphenyltin complex (1.3)

IR (cm^{-1}): 3336 (N-H str.), 1697 (Amide C=O), 1485 (C=C), 1581 (N-H bend), 1211 (C-N), 3429 (O-H), 1346 (N-O sym.), 1519 (N-O asym.), 420 (Sn-O) 549 (Sn-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.126-7.344 (1H each, s, 2,6-CH), 5.82 (1H each, s, 3,4,5-OH), 6.668 (1H, s, CONH), 7.38-8.05 (1H, m, 11, 12, 13, 14-Aromatic H) 6.810 (1H, m, β, γ, δ Aromatic-H); Yield 87%; m.p. 80ºC; mol.wt. 613.158 (g/mol); dark yellow. Numbering of C-atom is given in scheme 6.

Spectral data of dimethyltin complex (1.4)

IR (cm^{-1}): 3336 (N-H str.), 1674 Amide C=O), 1477 (C=C), 1589 (N-H bend), 1211 (C-N), 3488 (O-H), 1330 (N-O sym.), 1523 (N-O asym.), 432 (Sn-O) 552 (Sn-N) ; ^{1}HNMR (DMSO, 300 MHz) δ 7.292-7.215 (1H each, s, 2,6-CH), 5.27 (1H each, s, 3,4,5-OH), 6.326 (1H, s, CONH), 6.32-8.26(1H, m, 11, 12, 13, 14-Aromatic H), 0.71 (3H, s, α-CH_3); Yield 88%; m.p. 65ºC; mol.wt. 488.95 (g/mol); brown. Numbering of C-atom is given in scheme 6.

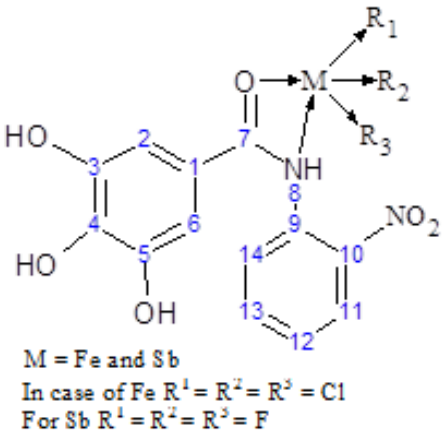


Scheme 4: Numbering scheme for zinc and copper complexes

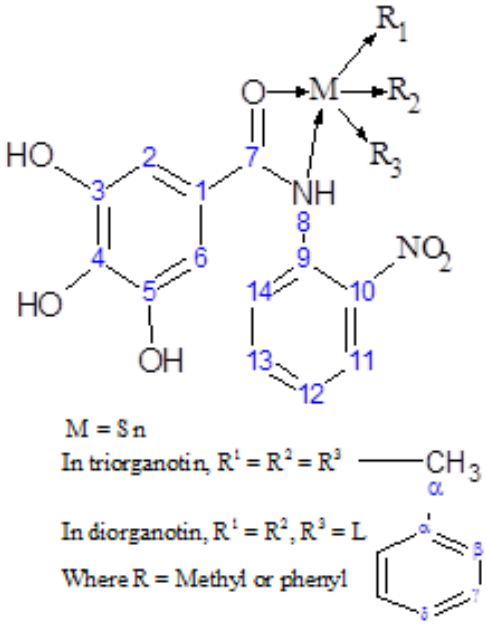
Spectral data of trimethyltin complex (1.5)

IR (cm^{-1}): 3336 (N-H str.), 1697 (Amide C=O), 1485 (C=C), 1581 (N-H bend), 1199 (C-N), 3429 (O-H), 1346

(N-O sym.), 1519 (N-O asym.) 420 (Sn-O) 573 (Sn-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.228-7.382 (1H each, s, 2,6-CH), 5.84 (1H each, s, 3,4,5-OH), 6.968 (1H, s, CONH), 7.255-8.140 (1H, m, 11, 12, 13, 14-Aromatic H), 0.68 (3H, s, α-CH_3); Yield 79%; m.p. 60ºC; mol.wt. 333.83 (g/mol) ; brownish yellow. Numbering of C-atom is given in scheme 6.



Scheme 5: Numbering scheme for iron and antimony complex



Scheme 6: Numbering Scheme for Tin

Spectral data for iron complex (1.6)

IR (cm^{-1}): 3275 (N-H str.), 1672 (Amide C=O), 1477 (C=C), 1558 (N-H bend), 1087 (C-N), 3412 (O-H), 1346 (N-O sym.), 1527 (N-O asym.), 449 (Fe-O), 536 (Fe-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.12-7.291 (1H each, s, 2,6-CH), 5.31 (1H each, s, 3,4,5-OH), 6.64 (1H, s, CONH), 6.93-7.84 (1H, m, 11, 12, 13, 14-Aromatic H); Yield 85 %; m.p. 65ºC; mol.wt. 416.97 (g/mol); dark green. Numbering of C-atom is given in scheme 5.

Spectral data for copper complex (1.7)

IR (cm^{-1}): 3379 (N-H str.), 1697 (Amide C=O), 1485 (C=C), 1573 (N-H bend), 1195 (C-N), 3552 (O-H), 1338 (N-O sym.), 1531 (N-O asym.), 451 (Cu-O) 505 (Cu-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.224- 7.268 (1H each, s, 2,6-CH), 5.76 (1H each, s, 3,4,5-OH), 6.227 (1H, s, CONH), 6.914-7.34 (1H, m, 11, 12, 13, 14-Aromatic H) 1.041 (3H, s, β-CH3); Yield 83%; m.p. 110ºC; mol.wt. 426.86 (g/mol); green. Numbering of C-atom is given in scheme 4.

Spectral data for zinc complex (1.8)

IR (cm^{-1}): 3275 (N-H str.), 1682 (Amide C=O), 1489 (C=C), 1558 (N-H bend), 1203 (C-N), 3429 (O-H), 1392 (N-O sym.), 1527 (N-O asym.), 432 (Zn-O) 538 (Zn-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.257-7.283 (1H each, s, 2,6-CH), 5.81(1H each, s, 3,4,5-OH), 6.132 (1H, s, CONH), 6.92-7.363(1H, m, 11, 12, 13, 14-Aromatic H), 1.860 (3H, s, β-CH3); Yield 86 %; m.p. 200ºC; mol.wt. 428.71(g/mol); light yellow. Numbering of C-atom is given in scheme 4.

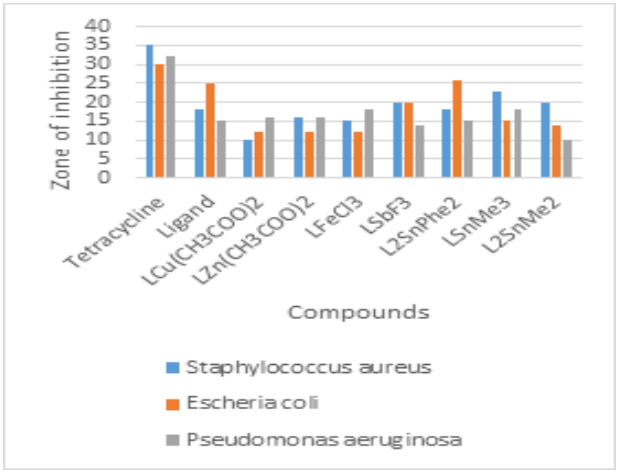


Fig. 1: Antibacterial activity chart of ligand and its metal complexes

Spectral data for antimony complex (1.9)

IR (cm^{-1}): 3289 (N-H str.), 1677 (Amide C=O), 1489 (C=C), 1581 (N-H bend), 1195 (C-N),3499 (O-H), 1346 (N-O sym.), 1527 (N-O asym.), 425 (Sb-O), 540 (Sb-N); ^{1}HNMR (DMSO, 300 MHz) δ 7.214-7.287 (1H each, s, 2,6-CH), 5.41 (1H each, s, 3,4,5-OH), 6.16 (1H, s, CONH), 6.93-7.343(1H, m, 11, 12, 13, 14-Aromatic H); Yield 86%; m.p. 130ºC; mol.wt. 348.85 (g/mol); greenish yellow crystalline. Numbering of C-atom is given in scheme 5.

DISCUSSION

^{1}H NMR Analysis

In HNMR, the expected peaks values were assigned by multiplicity as well as keeping in view intensity of observed peaks. Proton integration values were so helpful

in determining the expected chemical shift values of characteristics functional groups.

Existence of singlet at 6.443 ppm is a supportive evidence of formation of amide bond between carboxylic group (C-7) of gallic acid & 2-Nitro Aniline with replacement of OH group in ligand and more or less values were also seen in the HNMR spectra of all synthesized metal complexes.

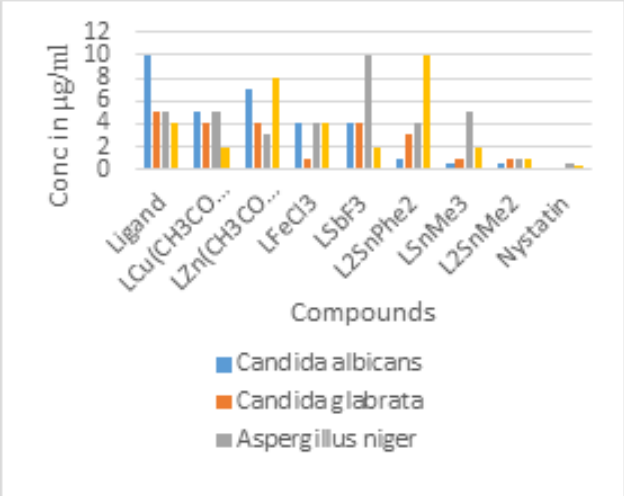


Fig. 2: Antifungal activity chart of ligand and its metal complexes.

In diorganotion & triorganation complexes where R= methyl, phenyl showed same or to some extent different resonating peak(s) at ppm scale in composition with that of ligand. Metal bonded methyl groups (M-CH_3) were de-shielded due to tin metal and appeared a singlet at 0.71 ppm (9H) and 0.68 ppm (6H) with varied intensity showing environmentally same methyl group for trimethyltin & dimethyl tin complexes respectively.

Phenyl (M-C_2H_5) in diphenyltin complex was somewhat deshielded and appeared at 6.810. Organometallic complexes (Fe and Sb) revealed deshielding behavior as compared to Sn-metal complex especially due to highly electronegative groups e.g., chloride and fluoride attached to iron and antimony. Little shielding effect on ligand was observed due to copper and zinc metals in their complexes, while methyl (β- CH_3) of acetate groups of metals appeared downfield at 1.04 and 1.86 respectively. The study of these spectra provided very useful information regarding structure determination of synthesized compounds.

IR analysis

The most significant band for ligand and its metal complexes were recorded by FTIR (Fourier transform infrared) spectrophotometer. Appearance of new N-H band in a range of 3275-3379 cm^{-1} (stretch), 1558-1589 (bend) and new C-N band in a range of 1087-1211 cm^{-1} are strong evidence for the attachment of amide group and formation of 2-nitro aniline derivative of GA (ligand). Also the –NH group is involved in coordination with metal complex. Appearance of new bands at range of 505-573 cm^{-1} for nitrogen metal (N-M) and for oxygen metal (O-M) at range 420-457 cm^{-1} in different metal complexes confirmed the development of new metal complexes. Appearance of two strong band bands in a range of 1512-1531 and 1338 to 1346 confirms that NO_2 group of aniline is attached and don't react with any moiety. As three hydroxyl groups are attached with the gallic acid and appearance of significant bands in a range of 3412 to 3698 confirms their attachment and no participation in chemical reaction. There is a strong band in range of 1620 to 1693 which ensures presence of aromatic moiety of starting compound (gallic acid).

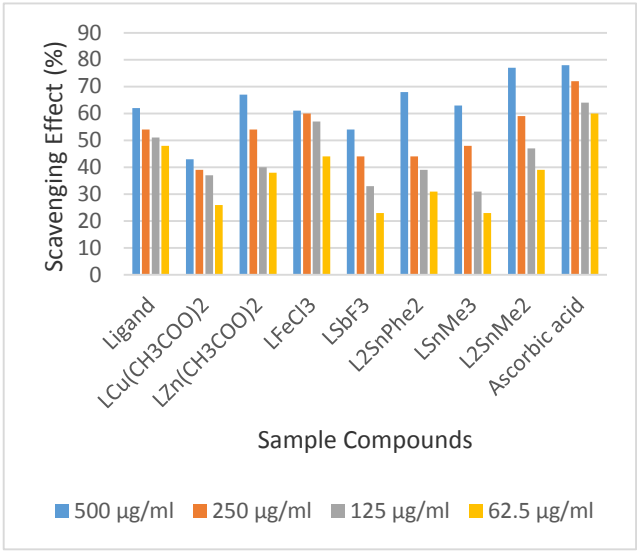


Fig. 3: Antioxidant activity chart of ligand and its metal complexes.

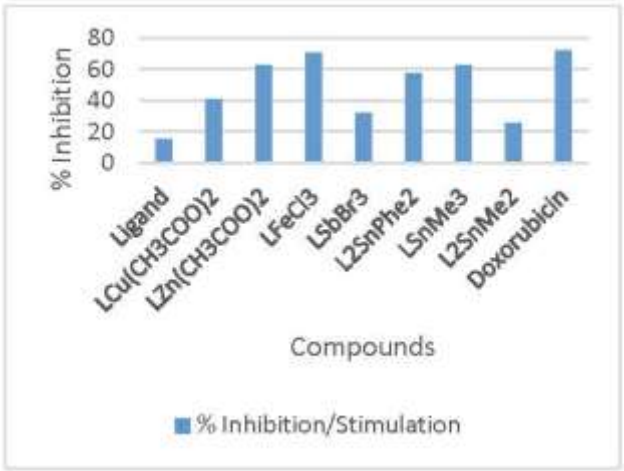


Fig. 4: Anticancer activity chart of ligand and its metal complexes.

Biological activities

Antibacterial activity

Agar diffusion method was used for determination of antibacterial activity. Metal complexes and ligand were screened *in vitro* against various bacterial strains e.g., gram negative and gram positive. Gram negative strain include *Escheria coli* while gram positive strains include *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Tetracyclin was used as standard drug. Results mentioned in fig. 1 shows that the ligand and metal complexes exhibit dominant activity against *Staphylococcus aureus, Escheria coli* and *Pseudomonas Aeruginosa.* More over other compounds like L_2SnPhe_2, and $LSnMe_3$ showed maximum antibacterial activity against *E. coli* and *Staphylococcus aureus* respectively (zone of inhibition 26mm and 23mm).

Antifungal activity

Ligand and metal complexes were screened against two fugal strains *in vitro,* using agar diffusion method. Using Sarbroud dextrose agar (SDA) media, fungal strains were cultured. Nystatin was used as a standard drug. Activity of ligand and its metal complexes against different strains is mentioned in fig. 2. $LSnMe_3$ and L_2SnMe_2 showed prominent activity against *Candida albicans* having MIC value of 0.3µg/ml and 0.5µg/ml respectively.

Antioxidant activity

Ligand and its metal complexes were screened for antioxidant activity by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) at different dilutions such as 62.5µg/ml, 125µg/ml, 250µg/ml and 500µg/ml. Ascorbic acid was used as standard. Antioxidant activities of ligand and its metal complexes in terms of mg/ml at different dilutions are shown in fig. 3. It is observed that ligand and all metal complexes has ability to donate its hydrogen to DPPH. Ligand shows promising results having 48% scavenging effect at 62.5 µg/ml dilution.

Anticancer activity (HeLa cell lines)

Cytotoxic activity of ligand and its metal complexes were evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. Results are shown in fig. 4. Iron, zinc and $LSnMe_3$ complexes of ligand showed prominent activity against HeLa cell lines having 61.17%, 62.43% and 62.4% respectively. While L_2SnPhe_2 complex of ligand showed more activity than all other having 70.1% inhibition.

CONCLUSION

In present research work, our successful efforts are the synthesis of novel compounds. 2-nitro aniline derivative of 3, 4, 5-trihydroxybenzoic acid and its metal complexes have been synthesized by the formation of amide bond between 3, 4, 5-trihydroxybenzoic acid and 2-nitro aniline. Characterization of synthesized compounds was carried out by several analytical techniques. In IR analysis, appearance of new N-H bands in the range of 3275-3336 cm^{-1} (str), 1558-1589 cm^{-1} (bend) justified the structure of ligand and formation of N-M and M-O bands in range of 505-573 cm^{-1} and 420-457 cm^{-1} respectively validated the synthesis of metal complexes.[1]HMNR spectral analysis interpretation also strengthens the structural elucidation of ligand due to presence of N-H peaks at 6.44 ppm. The slight upfield shift observed when metals were incorporated to the ligand which showed presence of metal in ligand. After performing antibacterial activity, compounds like L_2SnPhe_2, and $LSnMe_3$ shows prominent activity against *Esceria coli* and *Staphylococcus aureus* having 26mm and 23mm zone of inhibition respectively. Results for antifungal activity shows that $LSnMe_3$ and L_2SnMe_2 showed prominent activity against *Candida albicans* having MIC value of 0.3µg/ml and 0.5µg/ml respectively. For antioxidant activity, it is observed that ligand and all metal complexes has ability to donate its hydrogen to DPPH. Ligand shows promising results having 48% scavenging effect at 62.5 µg/ml dilution. For anticancer activity, Iron, zinc and $LSnMe_3$ complexes of ligand showed prominent activity against HeLa cell lines having 61.17%, 62.43% and 62.4% respectively. While L_2SnPhe_2 complex of ligand showed more activity than all other having 70.1% inhibition.

REFERENCES

Borges A, Ferreira C, Saavedra MJ and Simoes M (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.*, **19**(4): 256-265.

Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Ieri F and Romani A (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini-Rev. Med. Chem*, **8**(12): 1179-1187.

Cook JD, Reddy MB and Hurrell RF (1995). The effect of red and white wines on nonheme-iron absorption in humans. *Am. J. Clin. Nutr,* **61**(4): 800-804.

de Mejia EG, Chandra S, Ramírez-Mares M and Wang W (2006). Catalytic inhibition of human DNA topoisomerase by phenolic compounds in *Ardisia compressa* extracts and their effect on human colon cancer cells. *Food Chem. Toxicol*, **44**(8): 1191-1203.

Fazary AE, Taha M and Ju YH (2008). Iron complexation studies of gallic acid. *J. Chem. Eng. Data,* **54**(1): 35-42.

Kratz JM, Andrighetti-Fröhner CR, Kolling DJ, Leal PC, Cirne-Santos CC, Yunes RA, Nunes RJ, Trybala E, Bergström T and Frugulhetti IC (2008). Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl gallate. *Mem. Inst. Oswaldo. Cruz.*, **103**(5): 437-442.

Kubola J and Siriamornpun S (2008). Phenolic contents and antioxidant activities of bitter gourd (*Momordica*

charantia L.) leaf, stem and fruit fraction extracts *in vitro*. *Food Chem*, **110**(4): 881-890.

Kulisic T, Radonic A, Katalinic V and Milos M (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem.,* **85**(4): 633-640.

Lu Z, Nie G, Belton PS, Tang H and Zhao B (2006). Structure activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochem. Int.,* **48**(4): 263-274.

Madhavi D, Singhal R and Kulkarni P (1995). Technological aspects of food antioxidants. Food Sci. Tech. Marcel Dekker, New York, USA, pp.159-266.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **65**(1-2): 55-63.

Polewski K, Kniat S and Slawinska D (2002). Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies. *Curr. Top. Biophys.,* **26**(2): 217-227.

Rajalakshmi D and Marasimhan S (1995). Food Antioxidants: Sources and Methods ot Evaluation. Food Antioxidants: Technological: Toxicological Health Perspectives, CRC Press, pp.65.

Seo DJ, Lee HB, Kim IS, Kim KY, Park RD and Jung WJ (2013). Antifungal activity of gallic acid purified from *Terminalia nigrovenulosa* bark against *Fusarium solani*. *Microb Pathog*, **56**: 8-15.

Shahid K, Ali S and Shahzadi S (2009). The chemistry, properties and characterization of organotin (IV) complexes of 2-(N-naphthylamido) benzoic acid. *J. Coord Chem*, **62**(17): 2919-2926.

Shahid K, Shahzadi S and Ali S (2008). Spectroscopic studies of biologically active organotin (IV) derivatives of 2-[N-(2, 4, 6-tribromophenylamido] propanoic acid. *J. Iran Chem. Soc.,* **5**(4): 579-587.

Shahid K, Shahzadi S, Ali S and Bakhtiar M (2008). Characterization and antimicrobial activity of organotin (IV) complexes of 2-[(2, 6-diethylphenylamido)] benzoates and 3-[(2, 6-diethylphenylamido)] propanoates. *Turk. J. Chem.,* **32**(3): 333-353.

Shahzadi S, Shahid K, Ali S and Bakhtiar M (2008). Characterization and antimicrobial activity of organotin (IV) complexes of 2-[(2, 6-diethylphenylamido)] benzoates and 3-[(2, 6-diethylphenylamido)] propanoates. *Turk. J. Chem.,* **32**(3): 333-353.

Uozaki M, Yamasaki H, Katsuyama Y, Higuchi M, Higuti T and Koyama AH (2007). Antiviral effect of octyl gallate against DNA and RNA viruses. *Antiviral Res.,* **73**(2): 85-91.

Verma S, Singh A and Mishra A (2013). Gallic acid: molecular rival of cancer. *Environ. Toxicol. Pharmacol.,* **35**(3): 473-485.

Xia EQ, Deng GF, Guo YJ and Li HB (2010). Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.,* **11**(2): 622-646.