Research Article

Synthesis of Aniline Derivative of Ursolic Acid, its Metal Complexes, Characterization and Bioassay

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ABSTRACT

Present research work is an effort by synthesis of metal complexes (Cu, Zn, Sn, Sb and Fe) of ursolic acid derivative containing aniline moiety with substitution at C-28 (scheme 3) position of ursolic acid in order to enhance biological activities of metal complexes by coupling therapeutic values of ursolic acid and essential / transition metals as well. Antibacterial and antifungal activities of these complexes against some fungi and bacterial strains have been examined by agar well diffusion method, antioxidant effect of these complexes were studied by performing (2, 2-diphenyl-1-picryl-hydrazyl) DPPH assay. The structures of newly synthesized metal complexes were confirmed using IR, ^{1}H-NMR and ^{13}C-NMR spectral analysis.

Keywords: Aniline derivative of ursolic acid; metal complexes; Spectral characterization; antibacterial; antifungal; antioxidant activity

INTRODUCTION

Ursolic acid (UA, 3b-hydroxy-urs-12-en-28-oic acid), is a pentacyclic lipophilic triterpene acid, exist abundantly in the plant kingdom, an apple peel contains large quantities of ursolic acid and related compounds and is widely distributed in medicinal flora[1,2].

UA has potential to hold a wide spectrum of biochemical activities and pharmacological activities to intervene processes poorly modulated during cancer growth these include; inhibition of metastasis, tumorigenesis, angiogenesis, tumor promotion and induction of tumor cell differentiation[2-12]. Other biochemical activities are antiviral, anti-allergic, anti-inflammatory, antibacterial, antioxidant[13], hepatoprotective[14], anti-HIV and anti diabetic activities[15]. Among these attention-grabbing biological activities, the most astonishing and imperative property is the high cytotoxic activity[2-12].

Hydrogen/hydroxyl donor group at either C-3 or C-28 (scheme 3) position of ursolic acid provide best site for derivitazation as can be replaced by other functionalities, therefore in present research effort has been made to prepare the aniline derivative of ursolic acid by the replacement of OH group with aniline at C-28 (ligand) and further a series of metal complexes using different metals were synthesized from this aniline derivative of ursolic acid (ligand). Structure of these complexes and ligand were confirmed by spectral characterization technique i.e. IR, ^{1}H and ^{13}CNMR spectroscopy; also all complexes and ligand were evaluated *in vitro* for antibacterial, antifungal and antioxidant activities by reported method.

MATERIALS AND METHODS

Chemicals of high purity were used in synthesis of ligand and metal complexes. Organotin halides and other metal halides for synthesis of complexes were purchased from Sigma-Aldrich and were used without further purification, Ursolic acid having 98% purity was imported from China. Since the reactions were very sensitive to moisture therefore, the chemical used for the synthesis of metal complexes were dried in situ using standard procedures[16]. Other Chemicals like ethyl alcohol, methanol, acetone, acetonitrile, aniline and triethylamine were also of analytical grade.

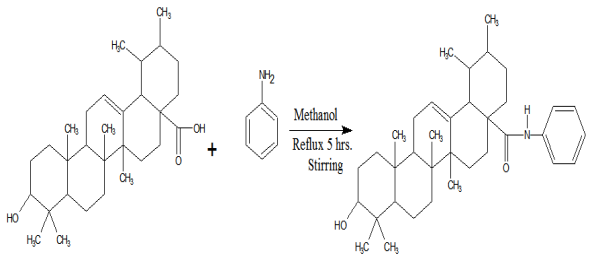
INSTRUMENTATION

Melting points were determined in a capillary tube using melting point (Gallenkamp MPA350.BM2.5) apparatus. The Infrared absorption spectra were recorded on Shimadzu FT-IR (IR-Prestige-21) Spectrophotometer by total reflectance method using ATR 8000A accessory. ^{1}H and ^{13}C-NMR spectra were measured on a Bruker ARX-300 MHz spectrometer at room temperature, with TMS as the internal standard.

PROCEDURE

General Reaction for Synthesis of Ligand

Reaction of Aniline with Ursolic acid



(3.1) Ursolic Acid + Aniline (3.2) Ligand

Scheme 1: Synthesis of Ligand.

Ursolic acid was used as the parent compound and the aniline derivative was prepared by the structure

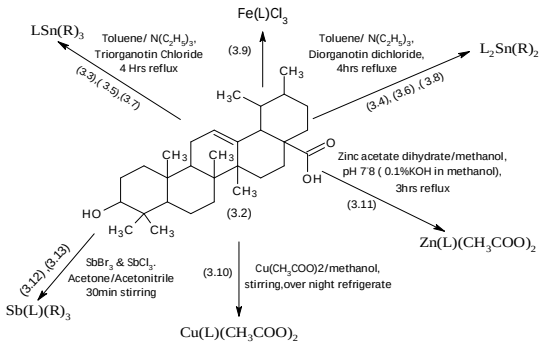
modification at the positions C-28 (scheme 3). The synthetic reaction is shown in Scheme 1.

General Reaction for Synthesis of Metal Complexes

Metal complexes (Sn, Sb, Cu, Fe, Zn) of aniline derivative of ursolic acid (Ligand) were synthesized with corresponding metal salts/halides i.e. tin halides (Triphenyltin Chloride, Diphenyltin dichloride, Tributyltin Chloride, Dibutyltin Dichloride, Trimethyltin Chloride, Dimethyltin Dichloride), Antimony halides (Antimony Trichloride, Antimony Tribromide), Copper (II) Acetate Monohydrate, Ferric (III) Chloride Anhydrous and Zinc Acetate Dihydrate. The synthetic reaction is shown in scheme 2.

General Procedure for the Synthesis of Amide Derivative of Ursolic Acid (ligand), Compound 3.2

Ursolic acid (1mmol) was suspended in methanol and treated with aniline (1mmol). The mixture was refluxed for 5 hours. The solvent was removed through rotary evaporator and the product was recrystallized from ethanol. A light yellow colored crystalline powder was obtained.



Scheme 2: Synthesis of metal complexes

General Procedure for the Synthesis of Metal Complexes of Ligand

General Procedure for the Synthesis of Triorganotin Derivatives of Ligand, Compound 3.3, 3.5, 3.7

1mmol of ligand was suspended in dry toluene and treated with triethylamine (1mmol). The mixture was refluxed for 3 hours. Triorganotin Chloride (1mmol) was added and the mixture was refluxed for 5-6 hours.

The solvent was removed through rotary evaporator and the product was recrystallized by using chloroform ($CHCl_3$)[17-21].

General Procedure for the Synthesis of Diorganotin Derivatives of Ligand, Compound 3.4, 3.6, 3.8

2mmol of ligand was suspended in dry toluene and treated with triethylamine (1mmol). The mixture was refluxed for 3 hours. Diorganotin Chloride (1mmol) was added and the mixture was refluxed for 5-6 hours. The solvent was removed through rotary evaporator and the product was recrystallized by using chloroform ($CHCl_3$)[17-21].

General Procedure for the Synthesis of Iron (III) Chloride complex of Ligand, Compound 3.9

Ligand (1mmol) and $FeCl_3$ (1mmol) were dissolved in equal volume of ethanol (25ml) separately, after mixing with stirring the reaction mixture was refluxed for 50 min. The resultant colored solution was left at room temperature. The product was obtained by filtration, washed with cooled absolute ethanol and recrystallized from methanol and dried under vacuum.

General Procedure for the Synthesis of Copper Acetate complex of Ligand, Compound 3.10

Copper complex was prepared using 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 mixture was adjusted to 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 mixture overnight in refrigerator. A green colored precipitates were obtained by filtration and dried over silica gel in a vacuum desicator.

General Procedure for the Synthesis of Zinc Acetate complex of Ligand, Compound 3.11

Ligand (1mmol) and zinc acetate 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 mixture was refluxed for 3 hours. A light yellow product was isolated by filtration and washed with methanol and dried under vacuum.

General Procedure for the Synthesis of Antimony Halide complexes of Ligand, Compound 3.12, 3.13

The complexes were prepared by mixing solution of ligand (1mmol) in 15ml of acetonitrile and solution of antimony halide (1mmol) in 15ml of acetone. The mixture was subjected to stirring for 30 minutes. Solution was filtered and kept at room temperature for crystallization. The crystallized product obtained was washed with methanol and dried.

Determination of Anti Bacterial Activity

Ligand and all the synthesized metal complexes were screened for antibacterial activity in vitro against different Gram negative and Gram positive strains by performing agar well diffusion method. The agar diffusion assay consisting of making 10ml aliquot of nutrient broth was inoculated with bacteria (test organism) and incubated at 37 ± 1 °C for 24 hours. 0.6ml of broth culture of test organism was added to molten agar cooled at 45 °C, mixed well and poured into a sterile petridish. Duplicate plates of each organism were prepared. The agar was allowed to solidify and required number of holes of 10mm was cut using a sterile cork borer, Agar plugs were removed.

Solution of ligand and metal complexes was prepared individually having concentration of 1mg/ml. 100µl of the test sample was dissolved in an appropriate solvent using a micropipette. Test sample was poured into appropriately labeled holes. Tetracycline (1mg/ml) solution was used as a positive control. The same volume of the standard antimicrobial agent tetracycline (1mg/ml) and the solvent (as control) were used. The plates were left at room temperature for 2 hours to allow diffusion of the sample and incubated at 37 ± 1 ºC for 24 hours faced upwards. Antibacterial activity was determined by measuring the zone of inhibition in millimeters[22].

Determination of Antifungal Activity

Ligand and their metal complexes were screened for antifungal activity in vitro against six (6) fungal strains using agar tube dilution method[23].

Sabouraud Dextrose Agar was prepared by mixing 4% Sabouraud Glucose Agar in distilled water with stirring and heating. Prepared media was poured into test tubes and autoclaved at 121 °C for 15 minutes. Autoclaved tubes were allowed to cool up to 50 °C. Solution of test samples was prepared individually having concentration of 20 µg/ml in sterile DMSO. Ketoconazole (20µg/ml) solution was used as a positive control. Using a micropipette transferred 100µl solution of test sample and positive control into different non-solidified sabouraud agar media tubes. Tubes were allowed to solidify in a slanting position at room temperature. Each tube was inoculated with a 4mm diameter piece of inoculums removed from a seven day old culture of fungal strains. All culture containing tubes were incubated at optimum temperature of 28-30 °C for 7-10 days. Humidity of the incubator was maintained about 40-50%. Cultures were examined at least twice weekly during the incubation. After 7-10 days of incubation the test tubes with no visible growth of microorganism were taken to represent the minimum inhibitory concentration (MIC) of the test sample which is expressed in µg/ml[23].

Determination of Anti Oxidant Activity

The free radical scavenging activity was measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. DPPH assay was performed according to the procedure described by Kulisic modified by Obeid, DPPH solution was prepared by dissolving 3.2mg in 100 ml of 82% methanol. DPPH solution (2800µl) was added to glass vial followed by the addition of 200µl of sample solution, leading to the final concentration of 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5 µg/ml (negative control). Mixture were shaken well and incubated in dark at temperature 25-28 °C for 1 hour. Absorbance was measured at 517nm by using UV-Visible Spectrophotometer (Shimadzu 1800). Ascorbic acid was used as positive control. The test was performed in triplicate.

Same procedure was then repeated with other fractions in order to determine their antioxidant activity[24,25].

Percentage inhibition of the DPPH radical by the samples was measured according to following formula and IC_{50} value was calculated by graphical method.

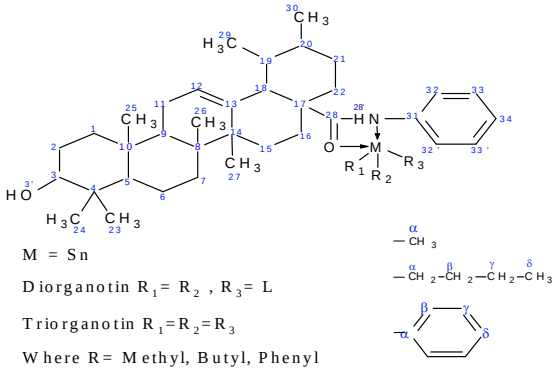
$$\text{Scavenging Effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where, **Ac** = Absorbance of negative control **As** = Absorbance of test sample.

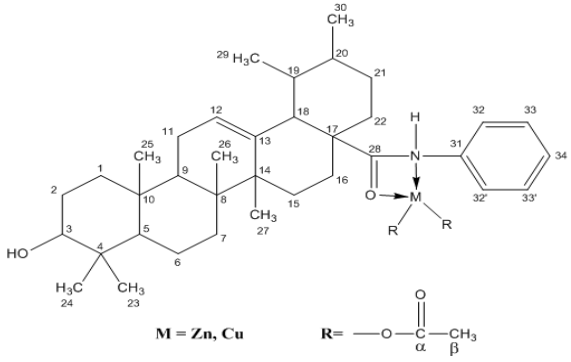
RESULTS AND DISCUSSION

Spectral Data

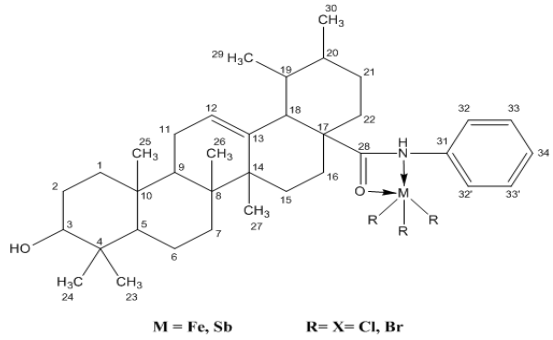
^{1}H, $^{13}CNMR$ spectrum of synthesized ligand and metal complexes were measured on 300MHz Bruker Avance multinuclear spectrometer. DMSO was used as solvent. The numbering scheme (3, 4a and 4b) for Ligand and their metal complexes is shown below:



Scheme 3: Numbering scheme for ligand and its metal complexes



Scheme 4(a): Numbering Scheme of Ligand and Metal (Cu/Zn) Complexes.



Scheme 4(b): Numbering Scheme of Ligand and Metal (Fe/Sb) Complexes

Spectral Data of Ligand (3.2)

IR (cm^{-1}): 3356 (N-H str.), 1643 (Amide C=O), 1610, 1458 (C=C), 1508 (NH bend), 1076 (C-N); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.52 (1H, br s, 28-CONH), 4.18 (1H, s, 3′-OH), 4.36-4.39 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 172.8 (28-C (28-CONH), 152.5 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C); Yield 85%; m.p. 175 ^{o}C; mol. wt. (g/mol) 532; Yellow crystalline.

Spectral Data of Triphenyltin Complex (3.3)

IR (cm^{-1}): 3360 (N-H str.), 1655 (Amide C=O), 1620, 1470 (C=C), 1510 (NH bend), 1076 (C-N), 443 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.61-0.74 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.15, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.66-4.15 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.60 (1H, br s, 28-CONH), 4.18 (1H, s, 3′-OH), 4.34-4.37 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 6.64-6.66 (1H, m, β, γ, δ Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 171.5 (28-CONH), 152.3 (31-C), 101.8 (32, 32′-C), 118.2 (33, 33′-C), 116.0 (34-C). 135.4 (α-C), 137.7 (β-C), 129.5 (γ-C), 128.6 (δ-C); Yield 85%; m.p. 180 ^{o}C; mol. wt; (g/mol) 882; White crystalline.

Spectral Data of Diphenyltin Complex (3.4)

IR (cm^{-1}): 3356 (N-H str.), 1650 (Amide C=O), 1615, 1470 (C=C), 1508 (NH bend), 1076 (C-N), 440 (Sn-N), 405 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.50-0.72(3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.16, 0.93-2.16 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.60 (1H, br s, 28-CONH), 4.17 (1H, s, 3′-OH), 4.32-4.375 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 6.83 (1H, m, β, γ, δ Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 171.1 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C). 131.5 (α-C), 129.5 (β-C), 128.6 (γ-C), 125.7 (δ-C); Yield 55%; m.p; 160 ^{o}C; mol. wt; (g/mol) 1336; Light yellow crystalline.

Spectral Data of Tributyltin Complex (3.5)

IR (cm^{-1}): 3354 (N-H str.), 1655 (Amide C=O), 1610, 1470 (C=C), 1510 (NH bend), 1076 (C-N), 460 (Sn-N), 426 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.74 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.98-2.50, 0.94-2.52 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.69-4.17 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.52 (1H, br s, 28-CONH), 4.20 (1H, s, 3′-OH), 4.33-4.35 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 1.38-1.52 (2H each, m, α, β, γ-CH_2), 1.21 (3H, s, δ-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 172.3 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C). 26.5 (α-C), 26.3 (β-C), 25.2 (γ-C), 8.9 (δ-C); Yield 86%; m; 185^{o}C; mol. wt; (g/mol) 822; White crystalline.

Spectral Data of Dibutyltin Complex (3.6)

IR (cm^{-1}): 3352 (N-H str.), 1650 (Amide C=O), 1610, 1465 (C=C), 1508 (NH bend), 1076 (C-N), 450 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.50-0.0.57 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.62, 0.97-2.62 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.55-4.15 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.63 (1H, br s, 28-CONH), 4.17 (1H, s, 3′-OH), 4.32-4.36 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 1.58-1.60 (2H each, m, α, β, γ-CH_2), 1.21 (3H, s, δ-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C), 27.4 (α-C), 26.5 (β-C), 25.6 (γ-C), 9.0 (δ-C); Yield 80%; m.p; 130 ^{o}C, mol. wt; (g/mol) 1296; Light yellow crystalline.

Spectral Data of Trimethyltin Complex (3.7)

IR (cm^{-1}): 3344 (N-H str.), 1641 (Amide C=O), 1610, 1465 (C=C), 1508 (NH bend), 1076 (C-N), 475 (Sn-N), 430 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.64-0.74 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.16, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.66-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.51 (1H, br s, 28-CONH), 4.18 (1H, s, 3′-OH), 4.34-4.38 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 0.89 (3H, s, α-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 173.1 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.0 (33, 33′-C), 115.9 (34-C), 8.9 (α-C); Yield 70%; m.p. 190 ^{o}C; mol. wt. (g/mol) 696; White crystalline.

Spectral Data of Dimethyltin Complex (3.8)

IR (cm^{-1}): 3354 (N-H str.), 1655 (Amide C=O), 1615, 1470 (C=C), 1508 (NH bend), 1076 (C-N), 450 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.60-0.74 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.16, 0.93-2.14 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.66-4.13 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.51 (1H, br s, 28-CONH), 4.16 (1H, s, 3′-OH), 4.31-4.36 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 1.28 (3H, s, α-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.0 (33, 33′-C), 115.9 (34-C), 8.9 (α-C); Yield 90%; m.p. 240 ^{o}C; mol. wt. (g/mol) 1212; White crystalline.

Spectral Data of Iron Complex (3.9)

IR (cm^{-1}): 3261 (N-H str.), 1700 (Amide C=O), 1600, 1460 (C=C), 1510 (NH bend), 1062 (C-N), 425 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.66 (1H, br s, 28-CONH), 4.25 (1H, s, 3′-OH), 4.36-4.40 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 171.1 (28-CONH), 152.6 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C), 8.9 (α-C); Yield 59%; m.p;120 ^{o}C, mol. wt. (g/mol) 694; Brown crystalline.

Spectral Data of Copper Complex (3.10)

IR (cm^{-1}): 3256 (N-H str.), 1631 (Amide C=O), 1616, 1459 (C=C), 1510 (NH bend), 1070 (C-N), 455 (Sn-N), 428 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.51 (1H, br s, 28-CONH), 4.18 (1H, s, 3′-OH), 4.36-4.39 (1H, m, 32, 32′, 33,

33′, 34 Aromatic-H), 1.28 (3H, s, β-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 150.2 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C), 175.2 (α-CO), 24.4 (β-C); Yield 82%; m.p. 165 ^{o}C; mol. wt. (g/mol) 713; Green crystalline.

Spectral Data of Zinc Complex (3.11)

IR (cm^{-1}): 3313 (N-H str.), 1650 (Amide C=O), 1620, 1465 (C=C), 1510 (NH bend), 1064 (C-N), 430 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.16 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.51 (1H, br s, 28-CONH), 4.18 (1H, s, 3′-OH), 4.36-4.39 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H), 1.26 (3H, s, β-CH_3); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 151.5 (31-C), 102.1 (32, 32′-C), 118.1 (33, 33′-C), 115.9 (34-C); 176.6 (α-CO), 25.2 (β-C); Yield 65%; m.p. 210 ^{o}C; mol. wt. (g/mol) 715; Light green crystalline.

Spectral Data of Antimony Trichloride Complex (3.12)

IR (cm^{-1}): 3354 (N-H str.), 1660 (Amide C=O), 1620, 1470 (C=C), 1510 (NH bend), 1076 (C-N), 447 (Sn-N), 420 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.18 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.85 (1H, br s, 28-CONH), 4.25 (1H, s, 3′-OH), 4.41-4.43 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 154.6 (31-C), 102.6 (32, 32′-C), 121.2 (33, 33′-C), 117.1 (34-C); Yield 72%; m.p. 140 ^{o}C, mol. wt. (g/mol) 760; Grayish white crystalline.

Spectral Data of Antimony Tribromide Complex (3.13)

IR (cm^{-1}): 3354 (N-H str.), 1650 (Amide C=O), 1610, 1470 (C=C), 1510 (NH bend), 1076 (C-N), 440 (Sn-N), 410 (Sn-O); ^{1}H-NMR (DMSO, 300 MHz) δ 0.62-0.78 (3H each, s, 23, 24, 25, 26, 27, 29, 30-CH_3), 0.97-2.17, 0.93-2.15 (2H each, m, 1, 2, 6, 7, 11, 15, 16, 21 and 22 α, β-CH_2), 0.65-4.17 (1H each, m, 3, 5, 9, 12, 18, 19, 20-CH), 6.78 (1H, br s, 28-CONH), 4.20 (1H, s, 3′-OH), 4.37-4.41 (1H, m, 32, 32′, 33, 33′, 34 Aromatic-H); ^{13}C-NMR (DMSO, 300 MHz) δ 172.5 (28-CONH), 153.7 (31-C), 102.5 (32, 32′-C), 121.1 (33, 33′-C), 117.0 (34-C); Yield 92%; m.p. 150 ^{o}C, mol. wt. (g/mol) 893; White crystalline.

^{1}H, ^{13}CNMR and IR Spectral Analysis

^{1}H NMR Analysis

^{1}H NMR spectra were recorded on 300 MHz Bruker Avance multinuclear NMR spectrometer using deutrated dimethylsulfoxide (DMSO) as solvent. The expected peaks values were assigned by multiplicity as well as keeping in view intensity of the observed peaks. Proton integration values were also helpful in determining the expected ppm values of characteristics functional groups.

Existence of singlet at 6.52 ppm is a supportive evidence of formation of amide bond between carboxylic group (C-28) of ursolic acid and aniline with the replacement of hydroxyl group in ligand and more or less same values were also observed in the ^{1}H NMR spectra of all the synthesized metal complexes.

In diorganotin and triorganotin complexes where R = methyl, butyl, phenyl showed same or to some extent different resonating peak(s) at ppm scale in comparison with that of ligand.

Methyl groups at C-23, 24, 25, 26, 27, 29 & 30) in ligand, di and trimethyltin complexes appeared as triplet at (0.62-0.78 ppm). Metal bonded methyl groups (Sn-CH_3) were deshielded due to tin metal and appeared as singlet at 0.89 ppm (9H) & 1.28 ppm (6H) with varied intensity showing environmentally same methyl group for trimethyltin & dimethyltin complexes respectively.

^{1}H NMR data suggested that dibutyltin complex α-CH_2 (methylene) appeared as triplet at 1.60ppm (4H, 2CH_2), β & γ-CH_2 (methylene) were slightly shielded and appeared at 1.58ppm (8H, 4CH_2) and δ-CH_3 (methyl) resonated at 1.21ppm as triplet (6H, 2CH_3) in comparison with tributyltin complex α-CH_2 (methylene) resonated as triplet at 1.52ppm (6H, 3CH_2), β & γ-CH_2 (methylene) appeared at 1.38 ppm (12H, 6CH_2) and δ-CH_3 (methyl) resonated at 1.21ppm as triplet (9H, 3CH_3).

The α-CH_3 (methyl) in trimethyltin resonating as singlet at 0.89 ppm (9H, 3CH_3) and dimethyltin resonating as singlet at 1.28 ppm (6H, 2CH_3) exhibited different behavior compared with the δ-CH_3 (methyl) resonating at 1.21 ppm (t, 9H, 3CH_3) and (6H, 2CH_3) of tributyl and dibutyltin complex respectively.

Phenyl (Sn-C_6H_5) in diphenyltin complex was somewhat deshielded as compared to triphenyltin complex and appeared at 6.83ppm and 6.64ppm respectively.

Organometallic complexes (Fe and Sb) showed deshielding behavior as compared to Sn-metal complex especially due to highly electronegative groups chloride and bromide attached to iron and antimony. Little shielding effect on ligand was observed due to zinc and copper metals in their complexes, while the methyl (β-CH_3) of acetate groups of metals appeared downfield at 1.26-1.28 ppm. The study of these spectra provided very helpful information for the determination of structure of the synthesized complexes.

^{13}C NMR Spectral Analysis

Synthesis of ligand was confirmed by the formation of amide bond between ursolic acid and aniline which is accompanied by the presence of peak at 172.8ppm for C-28[26].

Chemical shift values of C-31 and C-34 suggested that nitrogen of aniline ring exerted mesomeric effect which transferred electron density on ortho and para carbons C-32 and C-34, therefore shielding effect was observed.

In triphenyltin and diphenyltin complexes, tin metal bonded to phenyl ring shifted the phenyl ring carbon (C-α, β, γ, δ) slightly downfield (128.6-135.4 ppm) and

(125.7-131.5 ppm) respectively as compared to the ^{13}C chemical shift value of the benzene ring (128.5ppm)[27].

In methyl and butyl tin complexes their respective α and δ-carbon (methyl) resonated around 9.0 ppm with varied intensity. Methylene carbon in tributyl complex (α, β & γ-CH_2) showed peaks at 25.2-26.5ppm.

A slight downfield chemical shift and less intense peak at 25.6-27.4ppm in dibutyltin in comparison to tributyltin were observed.

Organometallic complexes (Fe and Sb) showed deshielding behavior (152.6-154.6 ppm) in comparison with ligand (152.5 ppm) at carbon (C-31) of phenyl especially due to highly electronegative groups chloride and bromide attached to iron and antimony.

Little shielding effects on ligand was observed due to zinc and copper metal complexes.

The α-carbon (COO) of acetate moiety bonded to metal appeared at 175.2 and 176.6 ppm for copper and zinc acetate complexes whereas the β-carbon resonated at 24.4 and 25.2 ppm respectively.

The study of these spectra provided valuable information for the structure elucidation and confirmation of the synthesized complexes.

Infrared Spectroscopy

The most significant bands were recorded by FTIR (Fourier Transform Infrared) spectrophotometer for Ligand and all complexes.

The absence of **O-H** band and appearance of new **N-H** band 3356-3360 cm^{-1} (Str.), 1508cm^{-1} (bend) and new carbon-nitrogen (**C-N**) bands (1062-1076) confirmed the attachment of aniline group and formation of aniline derivative of ursolic acid (ligand).

Also the -NH group was involved in coordination with metal complex.

Appearances of new bands (430-475 cm^{-1}) for nitrogen-metal (**N-M)** and (405-430 cm^{-1}) for oxygen-metal (**O-M)** in different metal complexes confirmed the development of new metal complexes.

Moreover in all complexes there has been predicted formation of four membered lactone ring by the appearance of C=O stretch band at ύ 1874 cm^{1}.

Biological Activity

Antibacterial Activity

Antibacterial activity data against different bacterial strains is mentioned in Table 1

Although both the Gram-negative and Gram-positive bacterial strains were affected by the metal complexes, the Gram-positive organisms were more susceptible to the antibacterial action of most of the metal complexes than were Gram-negative organisms.

Against Gram-negative group Antimony bromide and antimony chloride complexes (Table 1) showed the maximum activity for *E.Coli* and *S.typhi* respectively (zone of inhibition 39mm and 31mm).

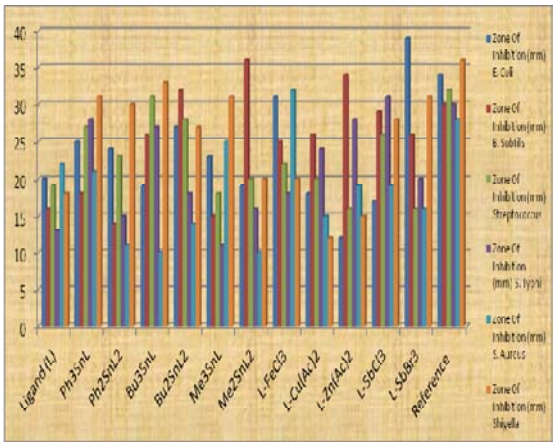


Figure 1: Antibacterial Activity Chart of Ligand and its Metal Complexes.

Against Gram-positive bacteria, the most active complexes were the Zn-complex, Fe-complex, dimethyl and dibutyltin complexes (Table 1).

Antifungal Activity

The antifungal activities of the ligand and metal complexes in terms of minimum inhibitory concentrations (MIC) and diameters of inhibition zones are reported in Table 2.

Dimethyltin, Copper and Zinc complexes showed highest effectiveness against *Bipole* and *M.canis* respectively with inhibition zone diameter of 100 mm.

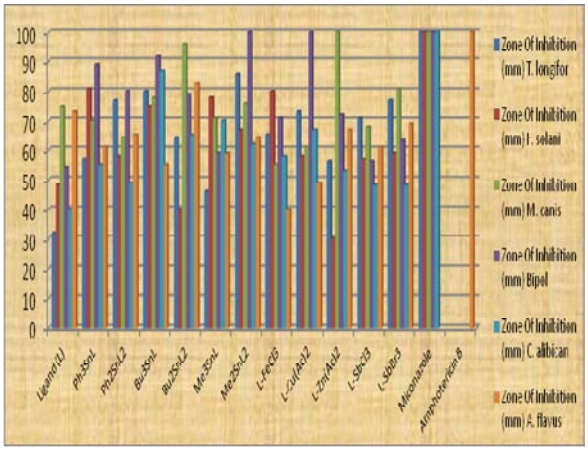


Figure 2: Antifungal Activity Chart of Ligand and its Metal Complexes.

Tributyltin and dibutyltin complexes exhibited higher antifungal activity against *Bipole* and *M.canis* respectively with inhibition zone diameter range of 92-96mm.

Dimethyltin complex, Di and triphenyltin complexes and iron complex showed high activity against *T.longifor*, *F.solani* and *Bipole* with inhibition zone diameter range of 80-89 mm.

Table 1: Antibacterial Activity data of Ligand and its Metal Complexes

Complex	Zone of Inhibition (mm)					
	E. Coli	*Bacillus Subtilis*	*Streptococcus*	*Salmonella Typhi*	*Staphylococcus Aureus*	*Shigella*
Ligand (L)	20	16	19	13	22	18
Ph_3SnL	25	18	27	28	21	31
Ph_2SnL_2	24	14	23	15	11	30
Bu_3SnL	19	26	31	27	10	33
Bu_2SnL_2	27	32	28	18	14	27
Me_3SnL	23	15	18	11	25	31
Me_2SnL	19	36	20	16	10	20
L-$FeCl_3$	31	25	22	18	32	20
L-$Cu(Ac)_2$	18	26	20	24	15	12
L-$Zn(Ac)_2$	12	34	16	28	19	15
L-$SbCl_3$	17	29	26	31	19	28
L-$SbBr_3$	39	26	16	20	16	31
Reference Drug (Tetracycline)	34	30	32	30	28	36

Table 2: Antifungal Activity data of Ligand and its Metal Complexes.

Complex	Zone Of Inhibition (mm)					
	Tricophyton Longifor	*Fusarium solani*	*Microsporum canis*	*Bipol*	*Candida alibican*	*Aspergillus Flavus*
Ligand (L)	32	48	75	54	40	73
Ph_3SnL	57	81	70	89	55	61
Ph_2SnL_2	77	58	64	80	49	65
Bu_3SnL	80	75	78	92	87	55
Bu_2SnL_2	64	40	96	79	65	83
Me_3SnL	46	78	71	59	70	59
Me_2SnL	86	67	76	100	62	64
L-$FeCl_3$	65	80	55	71	58	40
L-$Cu(Ac)_2$	73	58	61	100	67	49
L-$Zn(Ac)_2$	56	30	100	72	53	67
L-$SbCl_3$	71	57	68	56	48	61
L-$SbBr_3$	77	59	81	63	48	69
Reference Drug MIC µg/ml 100%	Miconazole	Miconazole	Miconazole	Miconazole	Miconazole	Amphotericin B

Antioxidant Activity

Antioxidant activity with IC_{50} value for ligand and synthesized metal complexes is mentioned in Table 3.

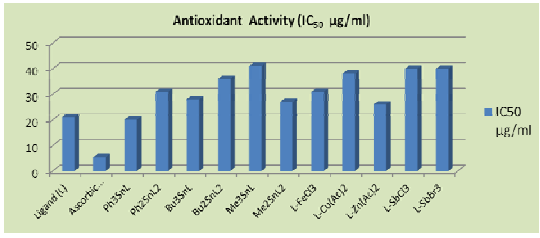


Figure 3: Antioxidant Activity Chart of Ligand and its Metal Complexes

Table 3: Antioxidant Activity data of Ligand and its Metal Complexes.

Complex	IC_{50} µg/ml
Ligand (L)	21
Ph_3SnL	20
Ph_2SnL_2	31
Bu_3SnL	28
Bu_2SnL_2	36
Me_3SnL	41
Me_2SnL_2	27
L-$FeCl_2$	31
L-$Cu(Ac)_2$	38
L-$Zn(Ac)_2$	26
L-$SbCl_2$	40
L-$SbBr_2$	40
Ascorbic acid	5.4

The antioxidant activities ranged from 21 to 41 µg/ml. No complex meet the antioxidant potential of the reference drug (Ascorbic acid), however amongst all compounds ligand displays the good antioxidant activity with the IC_{50} value of 21µg/ml.

CONCLUSION

From spectral characterization techniques it is concluded that the structure of ligand and all the synthesized metal complexes is consistent with that of their proposed structures and are biologically active as well. Furthermore it is concluded that Dimethyltin, Copper and Zinc complexes have highest antifungal activity against *Bipole* and *M.canis* respectively and are found as potent as the reference drug (Miconazole).

On the other hand antibacterial activity data shows that most of the metal complexes are most effective against Gram-positive organisms then Gram –negative. Gram-negative group is more susceptible to the antibacterial action of Antimony complexes and are more potent then the reference drug (tetracycline).

Zn-complex, dimethyl and dibutyltin complexes are more potent in antibacterial action then the reference drug (tetracycline) against Gram-positive bacteria *Bacillus Subtilis*, similarly iron complex is found to be highly active against *Staphylococcus aureus* and is more potent then the reference drug (tetracycline). Antioxidant data (Table 3) shows that all the synthesized metal complexes posses antioxidant activity to much lesser extent as compared to ligand and the reference drug as well.

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