**Journal of Pharmaceutical, Chemical and Biological Sciences**
ISSN: 2348-7658
CODEN: JPCBBG
Impact Factor (GIF): 0.701
Impact Factor (SJIF): 3.905
September- November 2017; 5(3):271-281
Published on: November 24, 2017

Research Article



# Antitumor, Cytotoxic and Antimicrobial Studies of A Novel Schiff Base, Ortho-Vanillin-(1,2-Ethylenediimine) Ortho-Hydroxyacetophenone and its Transition Metal Complexes

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**Received:**12 September 2017 **Revised:**29 October 2017 **Accepted:**02 November 2017

## ABSTRACT

The Schiff base, o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone(VEH), and its six metal complexes were synthesized, characterised and tested for their cytotoxic activities. The copper complex was found to have high $IC_{50}$ value, around 48 µ/ml. Daltons Lymphoma Ascites cell (DLA cell) induced solid and Ehrliche`s Ascites Carcinoma cell (EAC cell) induced ascites tumour models were used for antitumor studies of the compounds. Copper complex administrated at different concentrations in mice inhibited the solid tumour development and increased the mean survival rate and the life span of Ascites tumour enduring mice in a concentration dependent manner. The ligand and its metal complexes were screened against *C. albicans, C. Tropicalis* and *A. Flavus* fungi and *Pseudomonas aeruginosa,* (PTCC 1074) *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), and *Bacillus cereus* (PTCC 1015) bacteria to assess their potential antimicrobial activities. The results are quite promising. It is clear from the antifungal screening data that the metal complexes are more fungitoxic than the chelating agent itself. The bacterial screening results revealed that the free ligand has more sensitivity for gram-positive than gram-negative bacteria.

**Keyword:** o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone (VEH); Trypan blue exclusion metod; survival rate; Micro-both dilution method; cytotoxicity; anticancer; antimicrobial and antifungal

## INTRODUCTION

Compounds containing azomethine group (-CH=N-), known as Schiff bases derived from the condensation product of amines with carbonyl compounds [1-10]. Schiff bases and its metal compounds attract many scientists due to their wide range potential applications in cytotoxic, anticancer and pharmaceutical fields [11, 12]. e.g. as anticancer agent[13], as bactericides[14], antiviral agents[15] and fungicides[16,17]. The binding capacity of Schiff bases to metals has been widely studied. But, their relationship with each other has not been studied much. In Schiff base compounds, the imines nitrogen can act as an inter- or intra-molecular hydrogen-bond acceptor. H-bonding interactions are significant in their pharmaceutical industry applications. The nature and strength of the interactions between the molecules can influence the uptake of the medicin in the body. The actual role of metal complexes used as drugs were known only after the invention of *cis*-platin, ie., *cis*-[dichlorodiammine] platinum(II)[18]. Carbo-platin and *cis*-platin still have important roles in cancer chemotherapy [19, 20]. Since the invention of the anti-tubercular action of Schiff

base metal complexes, a gigantic study has been made on the field of pharmacology of these types of compounds[21]. Literature review reported that the metal complexes especially transition metal metals compounds of Schiff bases have better antitumour properties, when compared to free ligand [22]. They act in mammalian cells by encumber the enzyme, ribonucleotide reductase, is an essential in the preparation of DNA precursors[23]. Wang M and Wang L F *et al* [24] reported that the anticancer studies of metal compounds of thiosemicarbazone derived from 3-acetylumbelliferon. Among these complexes, Co(II) and Cu(II) exhibit better inhibitory effect compared to others.

The present work is an extension of our previous studies and is devoted to the preparation and identification of metal complexes with a Schiff base ligand synthesized from o-vanillin, o-hydroxyacetophenone and 1,2-ethylenediamine. The characterization was done based on elemental analysis, magnetic measurements, molar conductance, IR, $^1$H NMR and electronic spectral data. The ligand, o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone(VEH), and its copper complex were tested for their cytotoxic and antitumour activities. The ligand and its metal complexes were screened against *C. albicans, C. tropicalis, A. flavus,* and *A. niger* fungi and *Pseudomonas aeruginosa,* (PTCC 1074) *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), and *Bacillus cereus* (PTCC 1015) bacteria to evaluate their efficacious antifungal and antibacterial activities respectively.

## MATERIALS AND METHODS

The metal salts used in this study were BDH AnalaR quality. For the preparation of the HEH, o-Vanillin, o-hydroxyacetophenone and 1,2-ethylenediamine were used. Mainly chlorides, sulphates, nitrates and acetate of Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) were selected for the preparation of metal compounds. The commercially available solvents, ethanol, methanol, chloroform, DMF, DMSO etc. were taken for the preparation, extraction and recrystalization. All the solvents except E. Merk reagent grade were purified by standard method [25].

### Synthesis of the Ligand, O-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone: (VEH)):

A methanolic solution of o-Vanillin (0.025 mol in 25 ml) and o-hydroxyacetophenone (0.025 mol in 25 ml) were added to 1,2-ethylenediamine (0.025 mol) in minimum amount of methanol in a 250 ml round bottom flask with stirring[13]. The mixture was kept under reflux for two hours, and then the resultant yellow product was cooled in an ice bath. The product formed was filtered, washed a number of times with ethanol and finally with petroleum benzene and allowed to dried over anhydrous $CaCl_2$. Yellowish crystalline solid; yield 80 %; m. p: 142$^0$C; Solubility: DMSO, DMF; UV-Vis $\lambda_{max}$: 280 nm, 420 nm; IR: $\nu$ = 1612 cm$^{-1}$ (C=O), $\nu$ = 1684 cm$^{-1}$ $(C=N)_{azomethine}$, $\nu$ = 1518 cm$^{-1}$ $(C=N^{I})_{azomethine}$, $\nu$ = 3345 cm$^{-1}$ (-OH), $\nu$ = 3043 cm$^{-1}$ (-NH), $\nu$ = 3115 cm$^{-1}$ (Ar). (Fig.1)

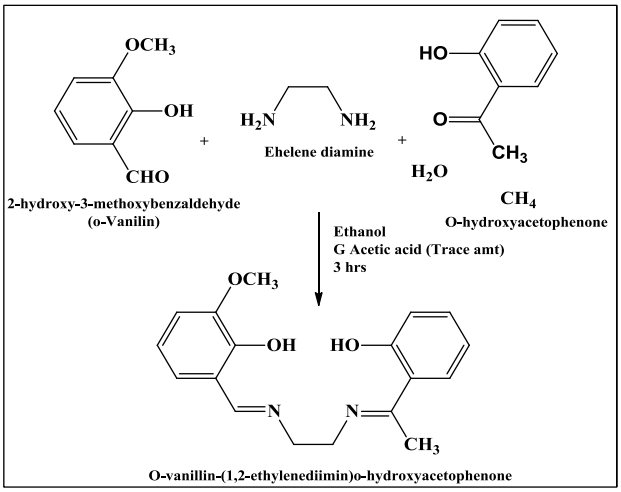


**Fig. 1: Synthesis of Ligand**

*****IUPAC name of the novel VEH Schiff base ligand : 2-((E)-((2-((E)-(1-(2-hydroxyphenyl)ethylidene)amino)ethyl)imino)methyl)-6-methoxyphenol

**Formation of metal complexes:**

The 0.005 mol of metal salts (Cr (III), Fe (III), Co (II), Ni (II), Cu (II) and Zn (II) ) in minimum amount of ethanol was added to DMSO solution of the ligand (0.005 mol in 20 ml) in dimethyl sulphoxide (DMSO) in 1:1 molar ratio and it was kept under reflux for about 4 h. It was then cooled and allowed to evaporate. After filtering, the solid complex obtained was washed with petroleum benzene and finally with ethanol and dried over anhydrous $CaCl_2$. (Yield: 70–80%, m.p = 250-350$^0$C) (Fig.2).

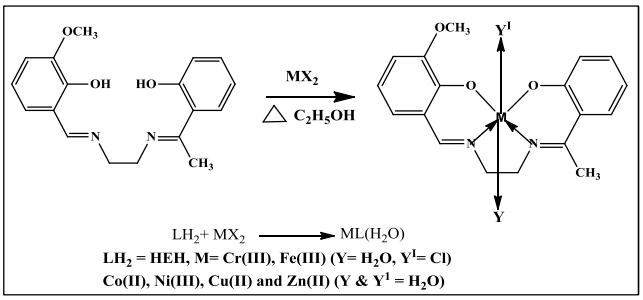


**Fig.2: Synthesis of metal complexes**

**Characterization of the Ligand, VEH**

The ligand (Fig.3a) and the complexes (Fig.3b) were characterized based on their elemental analysis, magnetic moment data, and IR, UV/Vis and $^1$H NMR spectral techniques.

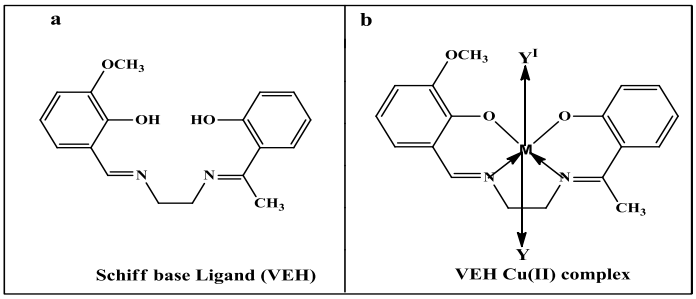


**Fig. 3: Structures of (a). VEH and (b). Cu(II) complex of VEH**

**Assessment of anticancer potential:**

The Cancer Institute (WIA) at Adayar, Chennai provided the essential EAC cell lines and Dalton's Lymphoma Ascites (DLA) and disseminated as transplantable tumours in the peritoneal cavity of BALB/C mice. The NCCS, is a national level biotechnology, tissue engineering and tissue banking research centre, Pune, India was supplied L929 (mouse lung fibro blast) cell line for our investigation.

The Swiss albino laboratory mice (20-26 g) were acquired from the Mannuthy small Animal Breeding Station, Thrissur, Kerala. They were stored in Amala Cancer Research Centre animal house, Trissur Kerala and provided standard condition of humidity and temperature with standard food (mouse chow) and water *ad libitum*. Institutional Animal Ethics Committee (IAEC) were given the prior permission for the all animal experiments in the present investigation and carried out strictly according to the strategy of CPCSEA established by the Animal Welfare Division, Government of India.

Mouse lung fibroblasts (L929 cells) were allowed to culture in DMEM medium supplemented with FBS (10% v/v), streptomycin (100 µg/ml) and penicillin (100 µ/ml) and stored at 37$^0$C in an incubator with 5% carbon dioxide. DLA and EAC cells were perpetuated in mouse (intraperitoneal cavity) were treated for the experiment.

**The synthesis of the drug:**

To 1 ml of the dimethylsulphoxide (DMSO) added 50 milligrams of the sample (Ligand and metal complexes) and dissolve. Using the solution *in vitro* studies was conducted. For conducting *in vivo* studies, 50 mg of the sample was first dissolved in 1 ml DMSO and it was further diluted with distilled water to the desired concentration.

**Trypan blue exclusion method:**

Using DLA cells, the compounds, to be tested, were investigated for short-term *in vitro* cytotoxicity studies. The cancer affected tumour cells for conducting the study aspirated from the peritoneal cavity of tumour bolstering mice where washed three times using PBS (phosphate buffered saline). Trypan blue exclusion method was used for the determination of Cell viability. The suspension of viable cell (1×106 cells in 0.1 ml) was added to test tubes enclosing various concentrations of the test samples and the volume was made up to 1 ml with PBS. The test tubes labelled as control consist of the cell suspension only. These samples and control to be analysed were allowed to incubate at $37^0$C for 3 hours. 0.1 ml of 1% Trypan blue was added to the cell suspension and placed for 2 to 4 minutes and loaded on a haemocytometer. It was observed that, the dead cells appeared in blue colour, due to the absorption blue stain of Trypan blue, while live cells did not absorb the dye. The number of each stained and unstained cells were estimated separately.

**Toxicity analysis Schiff base and its metal complexes:**

30 laboratory Swiss albino mice were categorized into 5 groups (6 mice/group). Group 1, 2, 3 and 4 were treated with 5mg/kg, 10 mg/kg, 15 mg/kg and 20 mg/kg respectively. The group 5 was treated as control. The drug was given to the mice once in a day by intraperitoneal injection and continued for 6 weeks. The mortality rate of the animals was noted[24].

**Effect of o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone copper complex**

**Survival rate of ascites tumour enduring Swiss albino mice;**

Mice (female, 40 – 56 days old) weighted 24–30 g were spitted into 5 groups having 6 animals per group. Viable EAC cells 106 in 0.1 ml of PBS were administrated by injecting on the peritoneal cavity of the animal. Group 1 and 5 were treated as control and standard (cyclophosphamide) respectively and group 2, 3 and 4 treated with 5mg/kg, 10 mg/kg and 15 mg/kg respectively.

Drug and standard drug cyclophosphamide were administrated by intraperitoneal injection to the animal from the first day of tumour induction [24]. The mortal rate of mice due to tumour encumbrance was recorded and percentage of increase in life span (ILS) was determined as, % ILS= (T-C/C) ×100, where T and C are mean survival of treated and control mice, respectively.

**On solid tumour development:**

Swiss albino mice (35 – 56 days old) weighing 24–28 g were splitted into five groups, each group composed of 6 animals for the above studies. DLA cells (0.1 ml of 106 cells per mouse) were administrated by injection in to the right hind leg of mice to induce tumour. Group 1 was treated as control animals. Copper complex of Schiff base VEH was given to the 2, 3 and $4^{th}$ groups for treatment. Group 5 was taken as standard animals and treated with standard drug cyclophosphamide. The tumour growth on the mice of each group was arbitrated by estimating the diameter of tumour volume in two perpendicular planes using a digital vernier calliper, starting from $7^{th}$ day of tumour growth up to $31^{th}$ day. The volume of tumour development was calculated using the equation, $V=4/3\pi r_1^2 r_2$, where $r_1$ is the minor diameter and $r_2$ is the major diameter [26].

**Antifungal activity:**

For the isolation of fungi, dilution plate method[27] was used. Selected and isolated fungi were maintained on potato dextrose agar plates at 4°C for further experimental work. The antifungal studies of the ligand (VEH), its complexes, fungicides (bavistin and emcarb) and the control DMSO (dimethyl sulfoxide) were screened using the plate poison technique. Seven day-old cultures of *C. Albicans, C. Tropicalis* and *A. Flavus* were used as test organisms. A stock solution of 500 g/ml was made by dissolving 50 mg of each compound in DMSO (100 ml). The sterilized medium with the stock solution was added into 90 mm sterile petri plates and kept for solidification. They were inoculated with a 5-mm actively growing mycelia disc and incubated at 27°C. After 72 h of inoculation, the percentage reduction in the radial growth diameter over the control was calculated. The growth was compared with dimethylsulfoxide as the control.

**Antibacterial activity**

In this study, we used four bacteria (two gram-positive bacteria and two gram-negative

bacteria). The standard strains of the following microorganisms were used as test organisms *Pseudomonas aeruginosa,* (PTCC 1074) *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), and *Bacillus cereus* (PTCC 1015). Bacterial isolates were developed in each medium for 24 h at 37°C. The inoculums density of each bacterial isolate was standardized with 0.5 McFarland turbidity standards. The suspension had a final inocolumn of 5 × 108 cfu/mL (colony-forming unit). Two methods, disc diffusion and micro-broth dilution methods were used to test antibacterial activity [28]. (Table.8)

**Disc diffusion method**

We prepared and sterilized the Müller Hinton agar medium (38 g Müller Hinton agar and 3 g agar agar in 1000 mL of distilled water). A small amount of each bacteria was placed on the side of the plate[29]. Using a sterile loop spread the bacteria in one direction from the starting site of inoculation. The plates were allowed to incubate at 37°C for 24 hours for bacterial growth. A bit of each bacteria was added in a sterile distilled water tube similar to 0.5 McFarland turbidity standard (the suspension had a final inoculum of 5 × 108 cfu/mL). The plates (with respect to the number of samples) were inoculated with bacteria by two sterile cotton swabs. The substance (0.02 g) was dissolved in 1 mL of DMSO. The sterile blank discs (Whitman no. 1 filter paper, 5 mm diameter) were dipped in 0.1 mL of each sample. The discs were placed on plates at specified intervals by sterile forceps. After an incubation period at 37°C for 24 hours, the diameter of each zone of inhibition was measured with a ruler (mm). The standards used for antibacterial measurement were Imipeneme (10 µg per disc), Ampicillin (10 µg per disc) and Chloramphenicol (30 µg per disc)[29]. To clarify any participating role of DMSO in the biological screening showed no activity against any bacterial strains. The test results are presented in Table 6. These results were confirmed by repeating the test three times using the same procedure conditions.

**Micro-broth dilution method for MIC**

The MIC (Minimum Inhibitory Concentration) is the lowest concentration of the test compound [12]. 13 sterile tubes containing 1 mL of the solution of Müeller nutrient broth medium were prepared. Each compound (0.02 g) was dissolved in 1 mL of DMSO. Then the first tube was filled with 1 mL of the test sample. 1 mL of the solution from the first tube was pipetted out and added to the second tube. Then, 1 mL of the solution from the second tube was pipetted out and added to the third tube. This process was repeated for all the 12 tubes. As a result, the concentration in each tube will be half of the previous tube. The extra solution (1 mL) from the $12^{th}$ tube was discarded. Thus, the $13^{th}$ tube acted as control bacteria. After 24 h of incubation at 37°C, a bit of one bacteria was dissolved in a sterile distilled water tube similar to 0.5 McFarland turbidity standard. A specified amount of bacterial suspension was added in all tubes except to the $12^{th}$ tube (as a control sample) until the concentration in all the tubes was 5 × 105 cfu/mL (Colony forming unit per millilitre) and incubate at 37°C. After 24 hours, MIC (Minimum Inhibitory Concentration) values of the substances were determined by the control tubes. MIC values of the ligand, VEH and its metal compounds are given in the Table.7.

**RESULTS**

**Short-term *in vitro* cytotoxic analysis of the ligand, VEH and its metal complexes**

The ligand, VEH and its Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) compounds exhibited striking cytotoxic activity against DLA cell line (Table 2). The Cu(II) VEH complex exhibited highest activity with an $IC_{50}$ (The concentration required for 50% death) value of 48 µg/ml.. (Table.2, Fig.4).

**Table 2: Percentage of cytotoxicity of ligand, VEH and its complexes**

| Concentration (µg/ml) | % of Cytotoxicity | | | | | | Ligand (VEH) |
|---|---|---|---|---|---|---|---|
| | Complexes | | | | | | |
| | Cr | Fe | Co | Ni | Cu | Zn | |
| 200 | 52 | 74 | 56 | 77 | 87 | 27 | 42 |
| 100 | 43 | 52 | 45 | 63 | 73 | 17 | 28 |
| 50 | 31 | 43 | 31 | 56 | 52 | 13 | 16 |
| 20 | 20 | 36 | 24 | 34 | 33 | 9 | 9 |
| 10 | 11 | 31 | 12 | 29 | 21 | 5 | 4 |

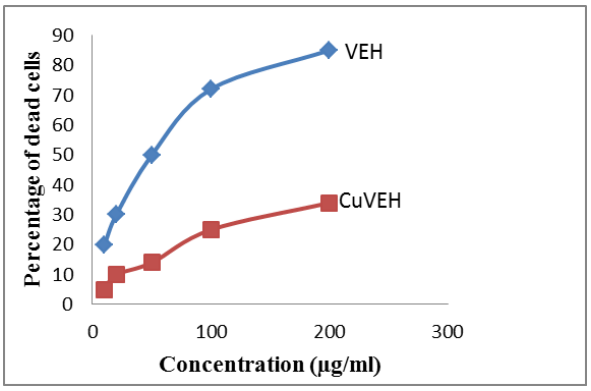


**Fig. 4: Cytotoxic action of VEH ligand and its Copper complex**

**Toxicity studies**

The findings of toxicity studies of Cu(II) VEH complex on 24 Swiss albino experimental mice of four groups, at four different concentrations (20, 15, 10 and 5 mg/kg) exhibited that 20 mg/kg was slightly noxious to the mice. Therefore, this concentration was abstained and only 15, 10 and 5 mg/kg were elected for *in vivo* studies.

**Action of Cu(II) complex of VEH on ascites tumour growth**

The tumour enduring Swiss albino mice of the control group survived for a period of 15 days. That group, treated by standard drug cyclophosphamide survived for 25.7 days. Those group treated by Cu(II) VEH complex at 15, 10 and 5 mg/kg concentrations raised the survival rate of mice by 17.4, 18.2 and 21.1 days, respectively (Table 3). Thus, it was found that the VEH of Cu(II) complex was effective in raising the average life span of the tumour enduring mice by 36.6, 17.9 and 13.6 % at 5, 10 and 15 mg/kg doses respectively (Table 4).

**Table 3: Effect of copper complex of VEH of survival rate of ascitis tumour enduring mice**

| Treatment (mg/kg) | Survival rate (Days) |
|---|---|
| **Control** | 15.3 |
| **15** | 17.4 |
| **10** | 18.2 |
| **05** | 21.1 |
| **Standard*** | 25.7 |

*cyclophosphamide (10)

**Table 4: Effect of copper complex of VEH on the life span rate of ascitis tumour enduring mice**

| Treatment (mg/kg) | Increase in average Life span (%) |
|---|---|
| **Control** | -- |
| **15** | 13.6 |
| **10** | 17.9 |
| **05** | 36.6 |
| **Standard*** | 67.5 |

* cyclophosphamide (10)

**Effect of Cu(II) VEH complex on reduction solid tumour volume**

Tumour volume of animals in the control group, enlarged by 3.022 $cm^3$ on 31$^{th}$ day, while treated with the Cu(II) VEH complex, there was a significant decrease of tumour volume. At 5 mg/kg, the volume was 1.428 $cm^3$, while at higher concentrations (15 and 10 mg/kg) the tumour volumes were found to be 1.845 and 1.741 $cm^3$, respectively. Treatment with standard drug, cyclophosphamide, reduction in tumour volume was 0.697 $cm^3$ (Table 5, Fig.5)

**Table 5: Effect of copper complex of VEH on the reduction of tumour volume**

| Dosage (mg/kg) | Observation (No: of days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | 10 | 13 | 16 | 19 | 22 | 25 | 28 | 31 |
| **Mean volume** | 0.093 | 0.702 | 0.890 | 1.431 | 1.972 | 2.343 | 2.690 | 2.984 | 3.022 |
| **15** | 0.081 | 0.511 | 0.592 | 0.683 | 0.835 | 0.885 | 0.993 | 1.121 | 1.845 |
| **10** | 0.079 | 0.481 | 0.461 | 0.791 | 0.814 | 0.924 | 1.025 | 1.343 | 1.741 |
| **05** | 0.076 | 0.464 | 0.485 | 0.643 | 0.715 | 0.795 | 0.947 | 1.013 | 1.428 |
| **Standard** | 0.081 | 0.215 | 0.215 | 0.249 | 0.305 | 0.401 | 0.491 | 0.513 | 0.697 |

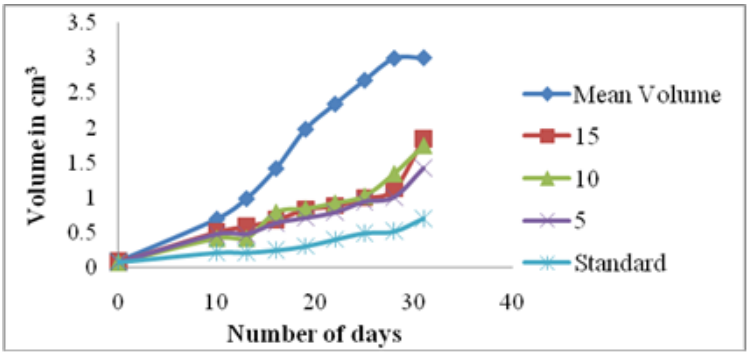


**Fig. 5: Effect of copper complex of VEH on solid tumour induced by Dalton`s lymphoma ascites cells**

**Antifungal studies**

The free Schiff base ligand, VEH and its metal compounds were screened against *C. albicans, C. Tropicalis* and *A. flavus* fungi and the results were quite promising. It was apprehensible from the antifungal screening study (Table.6) that the metal compounds of VEH were better fungitoxic than the ligand itself. It has been also observed that higher activity against A. Flavus and medium activity against *C. albicans* and *C. Tropicalis*. The complexes also exhibit species-dependent antifungal activity (Table 6).

**Table 6: Antifungal studies of ligand, VEH and its metal complexes**

| Compounds | Fungi , % Inhibition (growth diameter in mm) | | |
|---|---|---|---|
| | *C. albicans* | *C. Tropicalis* | *A. Flavus* |
| **Emcarb*** | (00)100 | (00)100 | ---- |
| **Bavistin*** | (00)100 | (00)100 | (00)100 |
| **DMSO (control)** | (21)29 | (28)33) | (32)40 |
| **Ligand** | (12)15 | (12)14 | (15)19 |
| **FeL** | (23)24 | (18)26 | (15)25 |
| **CoL** | (14)24 | (23)26 | (17)21 |
| **NiL** | (19)28 | (26)27 | (17)23 |
| **CuL** | (20)32 | (21)29 | (24)39 |

* Conventional fungicides.

**Antibacterial study:**

The VEH and its metal compounds were screened against *S. Aureus, B. Cereus, P. Aeruginosa* and *E.coli* bacteria to evaluate their potential antimicrobial activity. The antibacterial activity results (Table. 7) revealed that the free ligand, VEH has more potent for gram-positive than gram-negative bacteria. The biological activity of the transition metal VEH complexes follows the order Cu(II) > Ni(II) > Fe(III) > Co(II) > Cr(III). Thus the metal compounds were found to have higher biological activity than the parent Schiff base (Table 7 and 8) towards both gram-positive and gram-negative bacteria. This showed that the incorporation of metal ions in chelation can increase the antibacterial action of the parent organic ligand compounds.

**Table 7: Inhibition zones (mm) of complexes and ligand against bacterial strains**

| Compounds | Bacteria (D* mm) | | | |
|---|---|---|---|---|
| | ***S.aureus* (PTCC1112)** | ***B.cereus* (PTCC1015)** | ***E.coli* (PTCC1330)** | ***P.aeruginosa* (PTCC 1074)** |
| **L2H** | 8 | 7 | 6 | 6 |
| **FeL** | 14 | 12 | 10 | 11 |
| **CoL** | 8 | 11 | 11 | 8 |
| **NiL** | -- | 8 | 10 | 11 |
| **CuL** | 15 | 16 | 14 | 10 |
| **Ampicilin** | 14 | -- | 12 | -- |
| **Choloramphenicol** | 13 | -- | 14 | 8 |
| **Imipeneme** | 20 | 21 | 24 | -- |
| **DMSO** | -- | -- | -- | -- |

*(*D*) Diameter inhibition zone (in mm)

**Table 8: Minimum inhibition concentration, mg/ml**

| Compounds | Bacteria (D* mm) | | | |
|---|---|---|---|---|
| | ***S.aureus* (PTCC1112)** | ***B.cereus* (PTCC1015)** | ***E.coli* (PTCC1330)** | ***P.aeruginosa* (PTCC 1074)** |
| **L2H** | 2.50 | 5.00 | 2.50 | 5.00 |
| **FeL** | 0.15 | 2.15 | 1.50 | 2.50 |
| **CoL** | 0.50 | 0.31 | 1.25 | 2.50 |
| **NiL** | 0.61 | 1.25 | 0.62 | 1.25 |
| **CuL** | 0.65 | 2.50 | 0.62 | 1.25 |

**DISCUSSIONS**

Complexes of Schiff bases derived from diamines with ortho-vaniline and ortho-hydroxyacetopheneone and their complexes with transition metal ions are take up here. Metal ions (Chromium, Ironc, Cobalt, Nickel, Copper and Zinc) oordinated with this dianionic polydentate ligand, VEH Schiff base. When

transition metal ion and the ligand react in 1:1 molar ratio, there is chance of anion coordination as well.

The N-N-S and O-N-O donor systems are characteristic features in all metal complexes, has carcinostatic potency. We have carried the anticancer studies of o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone (VEH) and its metal compounds and we got interesting and promising results. *In vitro* cytotoxicity studies on VEH and its different metal compounds exhibited cytotoxicity against DLA cell lines. The Cu(II) VEH complex exhibited highest cytotoxicity with an interesting $IC_{50}$ value around 49 µg/ml.

From the present study, we concluded that the Cu(II) VEH compound is efficient for DLA-induced solid tumour and EAC-induced ascites tumour. Among the three concentrations (5 mg/kg, 10 mg/kg and 15 mg/kg body weights), 5 mg/kg body weights was more efficient than the other two, in both cases. *In vitro* cytotoxic and antitumour activities of the Cu(II) VEH compound, it can be suggest its potential use as an anticancer agent.

Bactericidal action of the ligand, VEH and their transition metal compounds were screened against different bacteria and the results are shown in Table 7. It has been suggested that the ligands with the O- and N-donor systems might have inhibited enzyme production. The complexation makes their diffusion through the lipid layer of spore membranes to the site of action easy and finally killing them. The deviation in the efficacious of diverse biocidal agents against different organisms depends on the cell impermeability. The inhibition zone diameter (mm/mg sample) data are concised in Tables 7, 8. A comparative study of the ligand, VEH and its metal complexes demonstrate that the later show higher antimicrobial activity than the free ligand, VEH. Such higher activity of the transition metal complexes can be elucidate on the basis of Tweedy's chelation theory[30,31]. Since there is a partial sharing of the positive charge of the metal ion with donor group in complex, chelation decreases its polarity. The delocalization of the π-electrons on the whole of the chelate ring supports this. The hydrocarbon acts as a lipophilic group[32] to drive the compound through the semipermeable membrane of the cell. The results revealed that the ligand has no promising activity against the bacteria.

## CONCLUSION

Coordination complexes of Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) with multidentate ONNO donor Schiff base ligand, o-vanillin-(1,2-ethylenediimine)o-hydroxyacetophenone (VEH) have been prepared and their physic-chemical properties have been studied. The ligand VEH synthesized by condensation reaction by taking 1:1:1 molar ratio of o-vanillin, o-hydroxyacetophenone and 1, 2-ethylenediamine. The present investigation of *in vitro* cytotoxic and antitumour properties of the copper complex ofo-vanillin-(1,2-ethylenediimine)o-hydroxy acetophenone (VEH) suggests its potential use as an anticancer agent. Antifungal and antibacterial study suggested that the VEH metal complexes of Fe(III), Co(II), Ni(II) and Cu(II) are better antimicrobial agents.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

## ACKNOWLEDGEMENT

The authors are thankful to Amala Cancer Research Centre, Trissur Kerala for providing necessary facility to conduct cytotoxic and antitumor studies. We are also thankful to SAIF Cusat, Kochin for providing the CHN analysis and spectral data.

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**Cite this article as:**
Subin Kumar K, Aravindakshan K K. Antitumor, Cytotoxic And Antimicrobial Studies of A Novel Schiff Base, Ortho-Vanillin-(1,2-Ethylenediimine)Ortho-Hydroxyacetophenone And Its Transition Metal Complexes . J Pharm Chem Biol Sci 2017; 5(3):271-281.