



# Microwave-Assisted Synthesis of Some New Mixed Ligand Ni(II) Complexes Its Characterization and Its Antimicrobial Study

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## **Authors' contributions**

This work was carried out in collaboration between both authors. Author JMP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SSP managed the analyses of the study. Both authors read and approved the final manuscript.

## **Article Information**

DOI: 10.9734/JPRI/2021/v33i31B31700

### Editor(s):

(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

### Reviewers:

(1) Ionita Gabriela, Romania.

(2) Dibyendu Banerjee, The West Bengal University of Health Sciences, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/69705>

**Original Research Article**

**Received 02 April 2021**

**Accepted 09 June 2021**

**Published 14 June 2021**

## **ABSTRACT**

Nickel(II) complexes of the type  $[Ni(P)(L).2H_2O]$  has been synthesized by using 2-amino-6-methyl pyrimidine-4-ol (HP) and amino acids (HL). Complexes synthesized by conventional as well as microwave methods. Results indicate that complexes synthesized by the microwave method are more efficient than the conventional method. In the microwave method preparation time is short (4-7 min.) compared to the conventional method (45 min.). Moreover, the microwave Assisted method gives a very high yield (90%) of the complexes. The microwave method used for the synthesis of the complexes has been found easier, convenient and eco-friendly. Complexes have been characterized by FTIR, Elemental analysis, Gouy experiment, Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA). Complexes have shown significant antimicrobial activity.

**Keywords:** Ni(II) complexes; 2-amino-6-methyl pyrimidine-4-ol; amino acids; microwave-assisted; antimicrobial activity.

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## 1. INTRODUCTION

Metal complexes have received substantial attention in the last many years due to their interesting characteristics in material science and biological systems [1]. Electrical and magnetic optoelectronic, properties of metals and metalloids can be tailored by their reaction with many ligands [2-7]. The importance of mixed ligand complexes in the biological process has been well studied [8]. It has been revealed that many of the metal complexes with amino acids have biological activity. Amino acids are the main chemical units of proteins that construct the structure for all living organisms and are important for various biochemical processes that support the processes of life in individuals. They are good chelating agents and can coordinate to transition metals through their amino or carboxylic groups. The amino acid-metallic ion interactions are found to be accountable for enzymatic activity and stability of protein structures. The metal-amino acid complexation is a vital field of study as they can be used as typical model systems to understand the metal-protein interaction in biological systems [9-20]. Antitumor activity of some mixed ligand complexes has also been reported [21]. The antibacterial and antifungal properties of a range of nickel(II) complexes have been evaluated against several pathogenic bacteria and fungi [22-28]. Nickel is also important for the healthy life of an animal. It associated with several enzymes. Nickel plays a role in physiological

processes as a co-factor in the absorption of iron from the intestine. Any Change in its concentration of metal leads to metabolic disorder [29-33]. With the discovery of biological importance of nickel, it is important to study its complexation with amino acids to understand more about functions of Ni complexes. Nickel(II) is an essential metal ion playing an active part in metalloenzyme hydrogenase, and it has a key role in the function of methyl-coenzyme reductase (MCR). Because of this, Ni(II) ion is a good target for binding by a ligand capable of inducing a desired activity [34-36]. Microwave-assisted Synthesis is a main branch of green chemistry. The important application of microwave-assisted synthesis in organic, organometallic and coordination chemistry continues to develop at an astonishing pace. Microwave-irradiated reactions under solvent-free or less solvent conditions offer reduced pollution, low cost and better yield, and simplicity in processing and handling. The main features of the microwave approach are shorter reaction times, simple reaction conditions and enhancement of products. A few researchers report on the Synthesis of metal complexes by microwave methods [37-39]. Therefore, it was considered to study the complexation and to determine the biological activity of nickel complexes. The present paper reports synthesis, characterization and antimicrobial studies of some new mixed ligand Ni(II) complexes by conventional method and microwave method.

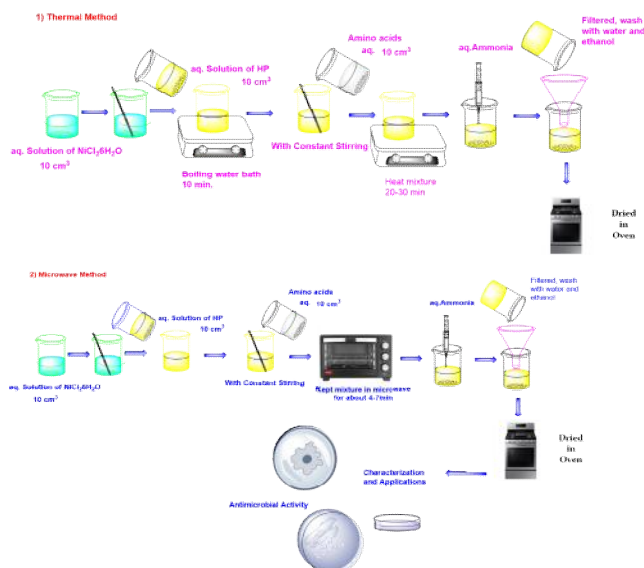


Fig. 1. Schematic representation for the synthesis of Ni(II) complexes

The microwave method gave very high yield in a shorter time. Ni(II) complexes prepared with 2-amino-6-methyl pyrimidine-4-ol (HP) as a primary ligand and amino acids (HL) such as L-leucine, L-lysine, L-tyrosine, L-aspartic acid, L-valine and L-phenylalanine as secondary ligands. The synthesized metal complexes have been characterized by elemental analysis and various physicochemical techniques such as molar conductance, magnetic susceptibility, electronic spectra, IR spectra and thermal studies.

## 2. EXPERIMENTAL SECTION

All the chemicals used in the present work were of the analytical grade (A.R.) and were procured from Aldrich, E. Merck and S.D. Fine. Metal salts were purchased from E. Merck and were used as received. All solvents used were of standard/spectroscopic grade. Analytical grade nickel(II) chloride hexahydrate was used as such without further purification. Amino acids such as L-leucine, L-lysine, L-tyrosine, L-aspartic acid L-valine and L-phenylalanine, were obtained from S.D. Fine Chemicals, Mumbai. In contrast, 2-amino-6-methyl pyrimidine-4-ol were obtained from Sigma Aldrich imported from U.S. Solvents ethanol, methanol Chloroform, DMF and DMSO whenever used were distilled and purified according to standard techniques [40-42].

### 2.1 Physical Measurements

Elemental analysis of the synthesized complexes were carried out on Thermo Finnigan Elemental Analyser model no FIASH EA 1113 series at IIT Mumbai. Content of metal was estimated complexometrically by standard protocol [43]. Fourier transforms infrared (FTIR) spectra of the prepared complexes were recorded in the spectral range 4000-400  $\text{cm}^{-1}$  on Perkin Elmer-spectrum FT-IR model no 1500 at IIT Mumbai. Ultraviolet (UV) spectra were recorded using a Perkin Elmer Lambda-950 UV-VIS spectrometer using DMSO as a solvent in the range of 200-800 nm. The molar conductance values were

measured in DMF ( $10^{-3}$  M) on an Equiptronics Autoranging Conductivity Meter Model No. EQ-667. Magnetic susceptibility measurements of complexes were carried out using Gouy balance at room temperature using  $\text{Hg}[\text{Co}(\text{NCS})]$  as a standard. Thermal Analysis (TG and DTA) were carried out in a controlled nitrogen atmosphere on a Perkin Elmer Diamond TG-DTA instrument.

### 2.1.1 Synthesis of Ni(II) complexes by a conventional method

Mixed ligand Ni(II) complexes were prepared from nickel(II) chloride hexahydrate, 2-amino-6-methyl pyrimidine-4-ol (HP) and different amino acids (HL) such as L-leucine, L-lysine, L-tyrosine, L-aspartic acid L-valine and L-phenylalanine. To an aqueous solution ( $10 \text{ cm}^3$ ) of Ni(II) chloride hexahydrate (237 mg, 1 mmol), an aqueous solution ( $10 \text{ cm}^3$ ) of 2-amino-6-methyl pyridine-4-ol (124 mg, 1 mmol) was added. The mixture was kept in a boiling water bath for 10 min. To this hot solution an aqueous solution ( $10 \text{ cm}^3$ ) of an amino acid (1 mmol) was added with constant stirring. The mixture was again heated in the water bath. The complexes were formed by increasing the pH of the reaction mixture by adding a diluted ammonia solution. The mixture was cooled and the solid complex obtained was filtered, washed with water and ethanol. The complexes prepared were dried in an oven.

### 2.1.2 Synthesis of Ni(II) complexes by microwave-assisted method

To an aqueous solution (10 mL) of nickel(II) chloride hexahydrate (237 mg, 1 mmol), an aqueous solution ( $10 \text{ cm}^3$ ) of 2-amino-6-methyl pyrimidine-4-ol (124 mg, 1 mmol) was added. To this hot solution, an aqueous solution ( $10 \text{ cm}^3$ ) of an amino acid (HL) (1 mmol) was added with constant stirring. The reaction mixture was kept in the microwave for about 4-7 min. The complexes were obtained by raising the pH of the solution by adding a diluted ammonia

**Table 1. Comparison between conventional and microwave method**

Sr. No.	Complex	Conventional Method		Microwave method	
		Time in min	% Yield	Time in min	% Yield
1	[Ni(P)(Leu).2H <sub>2</sub> O]	47	63	5	98
2	[Ni(P)(Lys).2H <sub>2</sub> O]	46	58	6	93
3	[Ni(P)(Tyr).2H <sub>2</sub> O]	45	56	5	92
4	[Ni(P)(Asp).2H <sub>2</sub> O]	46	65	6	97
5	[Ni(P)(Val).2H <sub>2</sub> O]	45	60	7	94
6	[Ni(P)(Phe).2H <sub>2</sub> O]	47	58	7	94

solution. The solution was cooled. The solid complex obtained was filtered and washed with water and ethanol. A comparison of these two methods is shown in table no. Table 1.

### 2.1.3 Antibacterial activity

#### Agar Cup Method

In the agar cup method, one compound can be tested against many organisms or a given organism against various concentrations of the same compound. The method was found to be useful for semisolid or liquid samples and was used in the current work. In this method, a plate of sterile nutrient agar with the desired test strain was transferred to a height of about 5 mm. It was solidified and a single cup of about 8 mm diameter was cut from the centre of the plate with a sterile cork borer. Afterwards, the cup was filled with the sample solution of known concentration and the plate was incubated at 37°C for 24 hrs. The level of growth inhibition from the edge of the cup was considered a measure of the given complex activity.

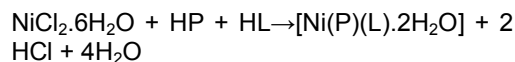
### 2.1.4 Antifungal

The primary fungi toxicity screening of the synthesized complexes was accomplished in vitro against the test fungi, *A. alternata* and *F. odum*, by the food poison technique [44-46]. The solution of complexes was prepared by dissolving them in DMF. Clotrimazole was used as a commercial antifungal agent and DMF served as a means of control. Potato dextrose agar medium was prepared using potato, dextrose, agar-agar, and DW and appropriate amounts of the complexes in DMF were added to potato dextrose agar to form 250, 125, 62.5 ppm solution of the complexes in the medium. The medium was poured into a set of two Petri plates under aseptic conditions in a laminar flow hood. When the medium in the vessels was solidified, mycelial discs of 0.5 cm in diameter-cut from the edge of the 7-day old culture were aseptically inoculated upside down in the Petri plates' centre. Petri plates were incubated at 25 ± 1°C until fungal growth in the control Petri plates was nearly completed. The mycelial growth of fungi (mm) in each Petri plate was measured diametrically, and growth inhibition (I) was calculated.

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of Metal Complexes

The synthesis of mixed ligand Ni(II) complexes may be represented as follows:



Where HP is 2-amino-6-methyl pyrimidine-4-ol and HL is an amino acid such as L-leucine, L-lysine, L-tyrosine, L-aspartic acid L-valine and L-phenylalanine. All the complexes are coloured. They are thermally solids and non-hygroscopic (Table 2), indicates a solid metal-ligand bond. The complexes are soluble in common organic solvents such as methanol, chloroform, DMF and DMSO. The elemental analysis data (Table 3) of metal complexes is consistent with their general formulation as 1:1:1, mixed ligand complexes of the type  $[\text{Ni}(\text{P})(\text{L}) \cdot 2\text{H}_2\text{O}]$ . The molar conductance values of the complexes in DMF at  $10^{-3}$  M concentration are low ( $< 1$ ), indicating their non-electrolytic nature [47].

### 3.2 Magnetic Studies

Magnetic moments of the Ni(II) complexes calculated from the measured magnetic susceptibilities after employing diamagnetic corrections. The observed  $\mu_{\text{eff}}$  values given in Table 3. Suggest the octahedral geometry for nickel complexes. The study reveals the paramagnetic nature of Ni(II) complexes.

### 3.3 Electronic Absorption Spectra

The electronic spectra of the metal complexes in methanol recorded in the U.V. region exhibit intra ligand and charge transfer transitions. The spectra indicate three transitions in the range 204-264 nm, 338 nm and 384-388 nm, which can be assigned to  $\pi \rightarrow \pi^*$ ,  $n \rightarrow \pi^*$  and the charge transfer transitions (LMCT) from ligands to the metal, respectively. The spectra of metal complexes in chloroform recorded in the visible region show transitions in the range 400-420 nm, ascribed to charge transfer transition. Two transitions around 560-600 nm and 830-850 nm may be ascribed to d-d transitions, which are the characteristic feature of transition metal complexes [48-51].

### 3.4 FTIR-Spectra

The FT-IR spectra of the metal complexes were recorded for KBr discs over the range 4000-400

$\text{cm}^{-1}$ . The Metal complexes have shown very complex FTIR spectra due to various bands with different intensities, making the interpretation difficult. However, an effort has been made to describe some of the importance bands based on reported infrared spectra of amino acids, 2-amino-6-methyl pyrimidine-4-ol and metal complexes [52-54]. An essential feature of these infrared spectra is the absence of band at  $\sim 3406 \text{ cm}^{-1}$  because of the O-H stretching vibration of HP. The assumption is that it leads to the complex formation that occurs by the removal of a proton from the hydroxyl group of HP moiety. A strong  $\nu(\text{CO})$  peak observed in the range  $1268\text{-}1216 \text{ cm}^{-1}$  in the complexes spectra indicates the 2-amino-6-methyl pyridine-4-ol moiety complexes coordinating through its nitrogen and oxygen atoms as an uni-negative bidentate ligand. The  $\nu(\text{NH}_2)$  mode observed at  $3240 \text{ cm}^{-1}$  in the free HP is found to shift to a lower wavenumber, i.e. in the range of  $3000\text{-}2920 \text{ cm}^{-1}$  indicates coordination through the tertiary nitrogen donor of HP. The in-plane and out-of-plane deformation modes observed at  $\sim 496 \text{ cm}^{-1}$  and  $\sim 691 \text{ cm}^{-1}$  respectively, in the spectrum of free HP are shifted to higher wavenumbers  $\sim 505 \text{ cm}^{-1}$  in the range,  $791\text{-}786 \text{ cm}^{-1}$  respectively, indicates coordination through the nitrogen atom of HP with the metal ion [55-57]. Broad peak observed in the region between  $3400\text{-}3500 \text{ cm}^{-1}$  due to asymmetric and symmetric O-H stretching modes and a weak band in  $1664 \text{ cm}^{-1}$  due to H-O-H bending vibrations, specifying the existence of a coordinated water molecule [58-60]. The  $\nu_{\text{asymmetric}}(\text{COO})$  I.R. value band of the free amino acids, i.e.  $\sim 1517 \text{ cm}^{-1}$  is shifted to a higher wavenumber, i.e.  $1686\text{-}1666 \text{ cm}^{-1}$  and the  $\nu_{\text{symmetric}}(\text{COO})$  mode observed at  $\sim 1426 \text{ cm}^{-1}$  in the spectra of the free amino acids is shifted to lower wavenumber, i.e.  $1162\text{-}1140 \text{ cm}^{-1}$ , in the spectra of complexes, indicates the coordination of the -COOH group via oxygen with the metal ion. The difference ( $\nu_{\text{asymmetric}} - \nu_{\text{symmetric}}$ ) in the range  $233\text{-}231 \text{ cm}^{-1}$  indicates that the M-O bond is purely covalent [61-62]. A new peak of weak intensity observed in the regions around  $600\text{-}590 \text{ cm}^{-1}$ , and  $510 \text{ cm}^{-1}$  may be ascribed to the N-O

and M-N vibrations. [63-64]. These vibrational bands are absent in the IR spectra of free HP and amino acids.

### 3.5 Thermal Studies

The TG and DTA studies of the complexes have been recorded in the controlled nitrogen atmosphere at a constant heating rate of  $10^\circ\text{C}/\text{min}$ . Thermal study on the mixed ligand nickel complexes in a controlled nitrogen atmosphere was carried out to understand the stages and temperature range of decomposition. The most probable decomposition pattern of the complexes is proposed based on the careful examination of TG and DTA curves. The thermo-analytical data were given in Table 4. The T.G. of the complexes shows that they are thermally stable. The complexes show the gradual loss in weight due to decomposition by fragmentation with increasing temperature. The complexes with L-Leucine, L-lysine, L-tyrosine, L-aspartic acid L-valine and L-phenylalanine show similar behaviour in TG and DTA studies. These thermograms show the loss in weight corresponding to two water molecules in the temperature range  $104\text{-}186^\circ\text{C}$ , followed by weight loss in the range  $286\text{-}522^\circ\text{C}$ , approximately equal to the algebraic sum of weight loss due to both amino acid and HP moieties [65-66]. The DTA of the complexes shows an endothermic peak in the range  $104\text{-}186^\circ\text{C}$ , indicating the presence of coordinated water molecules, and a single exothermic peak in the range  $286\text{-}522^\circ\text{C}$  demonstrates that there may be the simultaneous decomposition of amino acid and HP moieties. A constant weight plateau after  $700^\circ\text{C}$  indicates the completion of the reaction [67-70].

In Table 2. P represents the deprotonated primary ligand 2-amino-6-methyl pyrimidine-4-ol, whereas Leu, Lys, Tyr, Asp, Val, and Phe represent deprotonated secondary ligands L-leucine, L-lysine, L-tyrosine, L-aspartic acid, L-valine and L-phenylalanine.

**Table 2. Colour, molecular weight, decomposition temperature and pH of the nickel complexes**

Sr. No.	Complex	Empirical Formula	Molecular Weight	Colour	Decomposition Temperature ( $^\circ\text{C}$ )	pH
1	[Ni(P)(Leu).2H <sub>2</sub> O]	NiC <sub>11</sub> H <sub>24</sub> N <sub>4</sub> O <sub>6</sub>	367.02	Dark Blue	386	6.74
2	[Ni(P)(Lys).2H <sub>2</sub> O]	NiC <sub>11</sub> H <sub>25</sub> N <sub>5</sub> O <sub>5</sub>	366.03	Faint Blue	376	6.92
3	[Ni(P)(Tyr).2H <sub>2</sub> O]	NiC <sub>14</sub> H <sub>22</sub> N <sub>4</sub> O <sub>6</sub>	401.04	Dark Blue	384	6.86
4	[Ni(P)(Asp).2H <sub>2</sub> O]	NiC <sub>9</sub> H <sub>18</sub> N <sub>4</sub> O <sub>7</sub>	352.95	Dark Blue	390	6.86
5	[Ni(P)(Val).2H <sub>2</sub> O]	NiC <sub>10</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	336.99	Blue	386	6.98
6	[Ni(P)(Phe).2H <sub>2</sub> O]	NiC <sub>14</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	348.05	Faint Blue	386	6.94

**Table 3. Elemental analysis data, molar conductance and magnetic moment of nickel complexes**

Sr. No.	Complex	Elemental analysis found (calculated)				Molar conductance (Mhos cm <sup>2</sup> mol <sup>-1</sup> )	$\mu_{\text{eff}}$ (B.M.)
		% M	% C	% H	% N		
1	[Ni(P)(Leu).2H <sub>2</sub> O]	15.99 (16.57)	36.00 (37.02)	6.59 (7.41)	15.27 (16.68)	0.032	3.08
2	[Ni(P)(Lys).2H <sub>2</sub> O]	16.03 (17.47)	36.09 (37.18)	6.88 (7.51)	19.13 (20.05)	0.032	2.96
3	[Ni(P)(Tyr).2H <sub>2</sub> O]	14.64 (15.98)	41.93 (42.68)	5.53 (6.09)	13.97 (14.65)	0.033	3.17
4	[Ni(P)(Asp).2H <sub>2</sub> O]	16.63 (17.68)	30.63 (31.78)	5.14 (6.44)	16.63 (17.70)	0.030	3.08
5	[Ni(P)(Val).2H <sub>2</sub> O]	17.42 (18.48)	35.64 (36.87)	6.85 (7.54)	16.63 (17.70)	0.031	3.12
6	[Ni(P)(Phe).2H <sub>2</sub> O]	15.24 (16.35)	43.67 (44.39)	5.76 (6.89)	14.55 (15.78)	0.034	3.20

**Table 4. Thermal data of nickel complexes**

Sr. No.	Complex	Temperature range (°C)	% Weight loss due to water		Temperature Range (°C)	% Weight loss due to HP and Amino acid	
			Found	Calculated		Found	Calculated
1	[Ni(P)(Leu).2H <sub>2</sub> O]	107-186	9.71	10.40	286-501	76.58	72.37
2	[Ni(P)(Lys).2H <sub>2</sub> O]	108-167	10.30	9.75	288-521	81.78	74.32
3	[Ni(P)(Tyr).2H <sub>2</sub> O]	110-174	10.70	10.21	285-522	79.91	73.15
4	[Ni(P)(Asp).2H <sub>2</sub> O]	105-167	10.80	9.72	290-515	76.79	74.25
5	[Ni(P)(Val).2H <sub>2</sub> O]	104-175	9.90	10.08	292-519	73.62	73.44
6	[Ni(P)(Phe).2H <sub>2</sub> O]	106-170	10.80	9.72	284-520	79.91	73.15

**Table 5. MIC ( $\mu\text{g}/\text{mL}$ ) Data of Nickel complexes**

Sr. No.	Complex code	C. Albicans	S. diphtheriae	S. aureus
1	[Ni(P)(Leu).2H <sub>2</sub> O]	300	150	200
2	[Ni(P)(Lys).2H <sub>2</sub> O]	150	100	400
3	[Ni(P)(Tyr).2H <sub>2</sub> O]	350	150	400
4	[Ni(P)(Asp).2H <sub>2</sub> O]	300	200	400
5	[Ni(P)(Val).2H <sub>2</sub> O]	200	100	200
6	[Ni(P)(Phe).2H <sub>2</sub> O]	200	150	350
7	Doxycycline	2.0	1.5	1.5

**Table 6. Antifungal screening of the Ligand and Ni(II) complexes**

Sr. No.	Complex	Fungal inhibition (%) (Conc. in $\mu\text{g}/\text{ml}^{-1}$ )					
		Fusarium Odum			Alternaria alternata		
		250	125	62.5	250	125	62.5
1	Ligand	48.2	22.0	--	56.2	30.2	11.2
2	[Ni(P)(Leu).2H <sub>2</sub> O]	62.2	30.2	12.2	59.2	33.0	12.0
3	[Ni(P)(Lys).2H <sub>2</sub> O]	49.5	28.0	--	61.2	36.1	15.0
4	[Ni(P)(Tyr).2H <sub>2</sub> O]	51.1	23.0	11.2	57.0	34.2	12.0
5	[Ni(P)(Asp).2H <sub>2</sub> O]	54.4	32.0	12.3	63.2	45.2	18.4
6	[Ni(P)(Val).2H <sub>2</sub> O]	65.0	34.2	12.3	79.0	48.0	22.0
7	[Ni(P)(Phe).2H <sub>2</sub> O]	50.0	35.1	12.5	51.3	45.1	20.9
8	Clotrimazole (Std.)	94.0	74.0	42.0	98.0	86.0	46.0

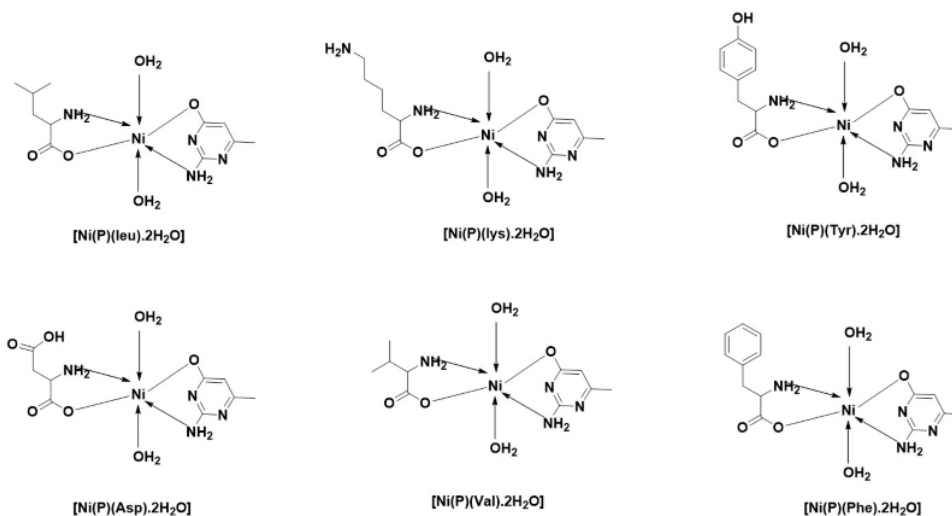


Fig. 2. Structures of Ni(II) complexes

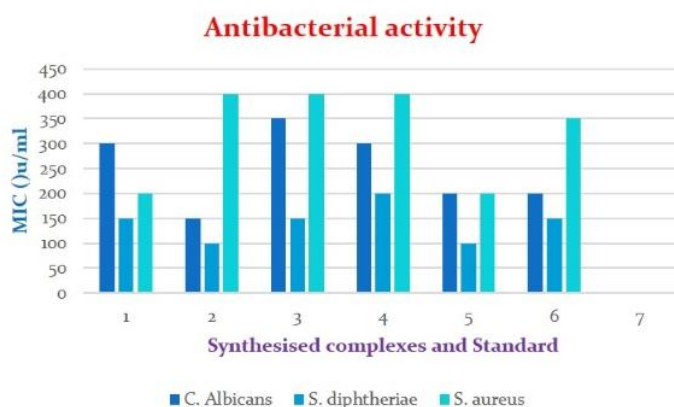


Fig. 3. Antibacterial activity of Ni(II) complexes

#### 4. CONCLUSION

We have enhanced the current method to synthesize some new Ni(II) complexes. Microwave-assisted method found to be energy efficient. Complexes were obtained in a shorter time (5-7 min) with high yield (90%). The synthesized Ni(II) complexes show higher decomposition temperature indicating a strong metal-ligand bond and electrical conductance studies display the non-electrolytic nature of the synthesized complexes. Magnetic studies reveal the paramagnetic nature of the synthesized complexes. The IR spectra show the bonding of the metal ion through N- and O- donor atoms of the two ligands. TG and DTA analysis support the presence of coordinated water molecules. Based on these outcomes, an octahedral

geometry may be proposed for synthesized nickel complexes. The antibacterial study shows that complexes are more active against *C. diphtheriae* than *C. Albicans*, and *S. aureus* compared with the standard antibacterial compound, doxycycline, the complexes show modest activity. The synthesized Ni(II) complexes show moderate activity against the selected strains of microorganisms. The antifungal activity of the Ni(II) complexes shows that complexes were highly potent than the free ligand against phytopathogenic fungi, *Fusarium odum* and *Alternaria alternata*.

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The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENT

The authors are thankful to Prof. V.D. Barhate, Principal of Changu Kana Thakur Arts, Commerce and Science College New Panvel (Autonomous) for administrative help and continuous encouragement and co-operation.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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