Synthesis, Characterization and Antimicrobial Activity of 2-[1-(2,4-Dihydroxyphenyl)Ethylene]Hydrazine Carboxamide and its Ni (II) Metal Complex

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Abstract

The synthesis of 2-[1-(2,4- dihydroxyphenyl)ethylene]hydrazinecarboxamide and its Ni(II) metal complex was achieved. 2, 4 dihydroxyacetophenone is synthesized by resorcinol with glacial acetic acid. This further treated with semicarbazide hydrochloride to form its semicarbazone. The dark blue Ni(II) metal complex was prepared by dissolving equimolar quantities of metal salt and Schiff base ligand in ethanol. All the target compounds were characterized by M.P, TLC and UV-visible and IR spectral data. Antimicrobial activities of these synthesized compounds were studied in sterile saline by Agar well diffusion method against *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa*bacteria. All the synthesized compounds have shown good to moderate antimicrobial activity.

KEYWORDS: Semicarbazone, metal complex, antimicrobial, diffusion method, IR spectroscopy

Introduction:

Semicarbazone moiety has chelating properties due to ONO donorgroup and it possess pharmaceutical activity.Due to electron rich hydrazine N atom present in complex with metal ion, it has interesting medical and nonmedical properties(Liu, Y et al 2018,Lashanizadegan et al, 2016). Now a day there is increasing interest in the discovery of new antibacterial agents because new strains of bacteria resistant to many currently available antibiotic reagents.Hydroxyacetophenones were used as starting material for the synthesis of chalcones (Kuzmin V, et.al.2005)flavones (Sawodny et al, 1978) and Schiff bases(Oshima S, et.al.(2002), Agarwala et al.(1992).Schiff bases of hydroxy aldehydes and ketones were widely used in co-ordination chemistry for the preparation of metal complexes (Hitoshi et al 1997, Punniyamurthy et al 1995). Schiff bases and their co-ordination compounds have been gained importance now-a-days as they are useful in biochemical, anti-cancer, anti-inflammatory (Fisher et al 1983)and antipyretic (Kleinberg et al, 1960)among others. Some of them have been used as complexing agent(Martell et al, 1952, Oxtoby et al, 2008) and powerful corrosion inhibitors(Gregory et al, 2004). A Schiff base of hydroxyacteophenone and its complexes has a variety of applications in biological, clinical, analytical and pharmacological areas(Miessler et al, 1999,Patai et al 1970). Earlier work has shown that some drugs showed increased activity when administered as metal chelates rather than as organic compounds(Brand et al 1943, John et al 1962) and that the co-coordinating possibility of hydroxyacetophenone has been improved by condensing with a variety of carbonyl compounds. Hence studying the biological activities of metal complexes is interesting. Thus the aim of the present work is to synthesize some new hydroxyacetophenones, its derivatives and their Ni metal complexes and to study theirantimicrobial activities. Their structures were elucidated on the basis of

TLC, MP, elemental analysis, UV-visible and IR spectral data. All the synthesized compounds screened for their biological activities by Agar well diffusion method.

Material and Methods:

In the present work the chemicals used were dry resorcinol, glacial acetic acid, powdered Zinc chloride, semicarbazide hydrochloride, anhydrous sodium acetate, ethanol etc. All these chemicals were purchased from Zen Scientific, Mumbai and were of AR grade. All these chemicals were used for synthesis.

Test Microorganism: The microorganism used in the present study were *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa.*. Microorganisms were maintained at 4^oC on nutrient agar slants.

The reagent 2-[1-(2,4- dihydroxyphenyl)ethylene]hydrazinecarboxamide and its Ni(II) metal complex were prepared as reported in the literature. The structure of the Schiff base is confirmed by using physical methods like melting point, TLC, elemental analysis, UV-visible and IR spectra. The newly synthesizedNi complex was characterized on the basis of microanalysis data, elemental analysis, UV-visible and IR.

Synthesis of 2,4- dihydroxyacetophenone

Briefly, the synthesis was carried out by dissolving freshly fused and powdered zinc chloride (0.24 mole) in 32 ml of glacial acetic acid by heating on sand bath. Dry resorcinol (0.2 mole) was added with stirring at 140^oC. The solution was heated until it just begins to boil and kept for 20 minutes at 150^0C. Dilute HCl (1:1) was added to the mixture and the solution was cooled to 5^0C. The separated product was filtered and washed with dilute HCl. The product was recrystallized from hot water. The reaction Scheme is given in Fig.1

fig.1 : Reaction scheme for the synthesis of 2,4- dihydroxyacetophenone

Synthesis of Semicarbazone Derivative:

1 gram of powdered semicarbazide hydrochloride was added 0.9 gram of anhydrous sodium acetate to 5ml of water and was warmed gently until a clear solution is appeared. A solution of 1gm of 2, 4 dihydroxyacetophenone in 5ml of ethanol was added. The mixture was warmed gently in a water bath for 15 mins. The Semicarbazone rapidly crystallizes. Finally, cool filter off the 2,4dihydroxyacetophenoneSemicarbazone, washed thoroughly with water and was drained. Recrystallised from ethanol.melting point is 201^oC. . The reaction Scheme is given in Fig.2

fig.2 : Reaction scheme for the synthesis of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Synthesis of Ni (II) Compex:

The Schiff base complexes under investigation were synthesized by mixing the Schiff base (0.1mm) in hot ethanolic solution, with hot ethanolic solution of $NiCl_2.6H_2O$. The reaction mixtures were refluxed in a water bath for 5hrs and their volumes were

reduced to 70% of its original volume and residue was left to stand overnight. The coloured products obtained were filtered by under suction, washed with ethanol. The product was recrystallized from ethanol. Their yields ranges from 50-55% the product obtained was greencoloured and melting point was 385^0 C. . The reaction Scheme is given in Fig.3

fig.3: Reaction scheme for the synthesis of nickel complex of of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Spectral Analysis: The structure of all synthesized compounds was confirmed by Elemental analysis,Uv-visible and IR Spectroscopic technique. The absorbance measurement was carried out on ShimarzuUv-visible 2100 spectrophotometer with 1 cm quartz cell. The IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. (Fig. 4-6)

fig 4: IR for 2,4- dihydroxyacetophenone

fig 5: IR for 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

fig 6: IR for nickel complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

Antimicrobial studies: Forantimicrobial activity the suspension of synthesized compounds and Ni (II) metal complex was made with sterile saline. In the present work the antimicrobial activities of the synthesized compounds have been screened against *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa*.

Preparation of test compounds: The solutions were prepared at a concentration of 1mg/µl for all the compounds.

Preparation of Plates and Microbiological assay: The antimicrobial evaluation was done by Agar well diffusion method[15,16] using Mueller Hinton Agar no.2 as the nutrient medium. The bacterial strains were activated by inoculating loop full of strain in 20 cm3 of nutrient agar and the same was incubated for 36 hrs. in an incubator at 37^oC. 0.1 cm3 of the activated strain was inoculated in Mueller Hinton agar. Mueller Hinton agar was kept at 45^oC and then poured in the petri dishes and allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 36 hrs. at 37^oC. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of these synthesized compounds.

Results and discussion:

2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazine carboxamide, its semicarbazone derivative and its Ni (II) metal complex are synthesized. The synthesized compounds were purified by recrystallization method. The purity of the compounds was checked by TLC using appropriate solvent systems. The physical and analytical parameters were checked. (Table1) The structure of all the synthesized compounds was confirmed by UV-visible and IR spectroscopic technique.

Table 1: physical and analytical data

Spectral Data:

2,4-dihydroxyacetophenone: IR(cm^{-1}, KBr) 3307(aro, O-H str), 3197.20(aro, C-H str), 1521.79(aro, C=C str), 1925.97(OH para sub), 1800(OH ortho sub), 1617.30 (C=O conj), 1143.96(aro, C-O str), 823.30(para sub), 729.16(ortho sub). (Table 2)

2,4-dihydroxyacetophenone semicarbazone: : IR(cm^{-1}, KBr) 3309(aro, O-H str), 3212.72(aro, C-H str), 1525.44(aro, C=C str), 1922(OH, para sub), 1805(OH ortho sub), 1688.10(C=O conj), 1143.83(aro, C-O str), 1213.08(C-N str), 1143.96(aro, C-O str), 839.28(para sub), 728.93(ortho sub). (Table 2)

Ni complex of semicarbazone derivative of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazine carboxamide: IR(cm^{-1}, KBr) 3131.30 (aro, O-H str), 2737.53(aro, C-H str), 1514.16(aro, C=C str), 1912.17(OH, para sub), 1681.41(C=O conj), 1156.59(aro, C-O str), 1284.67(C-N str), 859.77(para sub), 787.51(ortho sub), 514.28 (M-O stretch) (Table 2)

Table 2: spectral data

Antimicrobial Activity:

Microbial assay were carried out by agar well diffusion method. The bacterial strains were activated by inoculating loop full of strain in 20 cm3 of nutrient agar and the same was incubated for 36 hrs. in an incubator at 37^{o}C. The zone of inhibition in different bacterial strains i.e. *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa* against synthesized compounds shown in Table 3. Among the various bacterial strains maximum zone of inhibition was recorded in *S. aureus*and minimum zone of inhibition was observed in *M. luteus*and*P. aerug*(Table 3)

Table 3: antibacterial activity

Discussion:

The results indicate that inhibition depends on strain and structure. In compound I due to presence of only C=O it shows less inhibition. But due to presence of C=N imine group present in compoundIIinhibition increases compare to compound I. Also it contains a broad band at 3500-3400 and it is due to intermolecular hydrogen bonded –OH group, at 1600-1700 C=O (amide) strong absorption, Medium sharp absorption band is due to N-H stretching vibration at 3200-3100, 1675 due to tertiary amide strong absorption band 1600. Aromatic C-H band at 3000-2900. Physicochemical studies have been used in the elucidation of the geometry of the metal complexes with the purpose of throwing light on the structural aspects of newly synthesized Schiff base complexes. The newly synthesized ligand and its Ni (II) metal complex were characterized by elemental analysis, UV-visible, IR, TLC and repeated MPs determination studies. The susceptibility of the contain strains of bacteria towards the synthesized compounds was determined by measuring the size of inhibition diameter . Ni (II) complexes were highly effective against *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa* than their parent compound. It was also observed that antibacterial activity of these complexes for removing bacteria was fairly good.

Conclusion:

2,4- dihydroxyacetophenone ,its semicarbazone derivative and Ni (II) metal complex were synthesized. The structures of synthesized compound were supported by IR spectroscopic technique. The antibacterial screening of these compounds shows that inhibition depends on strain and structure. Presently, there is an emergence of multiple drug resistance to human pathogenic organism so there is increasing interest and need to develop new alternative antimicrobial drugs for the treatment of infectious diseases. The clinical isolated bacteria in this study were *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa*.. These isolates of bacteria caused high percentage of drug resistance. The antibacterial study showed that complexes are found to be more active against *M. luteus, B. subtilis, S. aureus, E. coli, P. aeruginosa*. Compared to standard antibacterial compound the complexes show moderate activity against the selected strains of microorganisms.

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fig.1 : Reaction scheme for the synthesis of 2,4- dihydroxyacetophenone

Reaction Scheme:

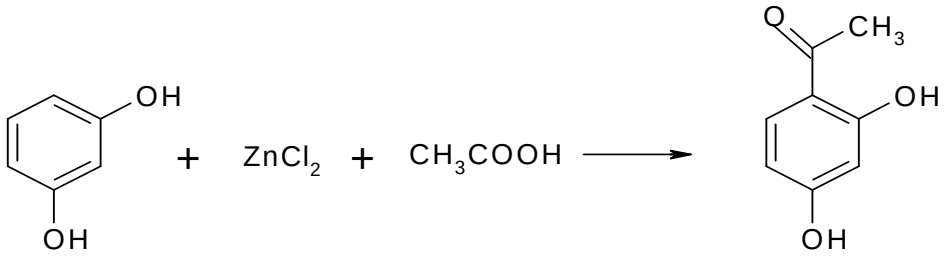

fig.2 : Reaction scheme for the synthesis of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide i.e. 2,4 dihydroxyacetophenonesemicarbazone,

Reaction Scheme:

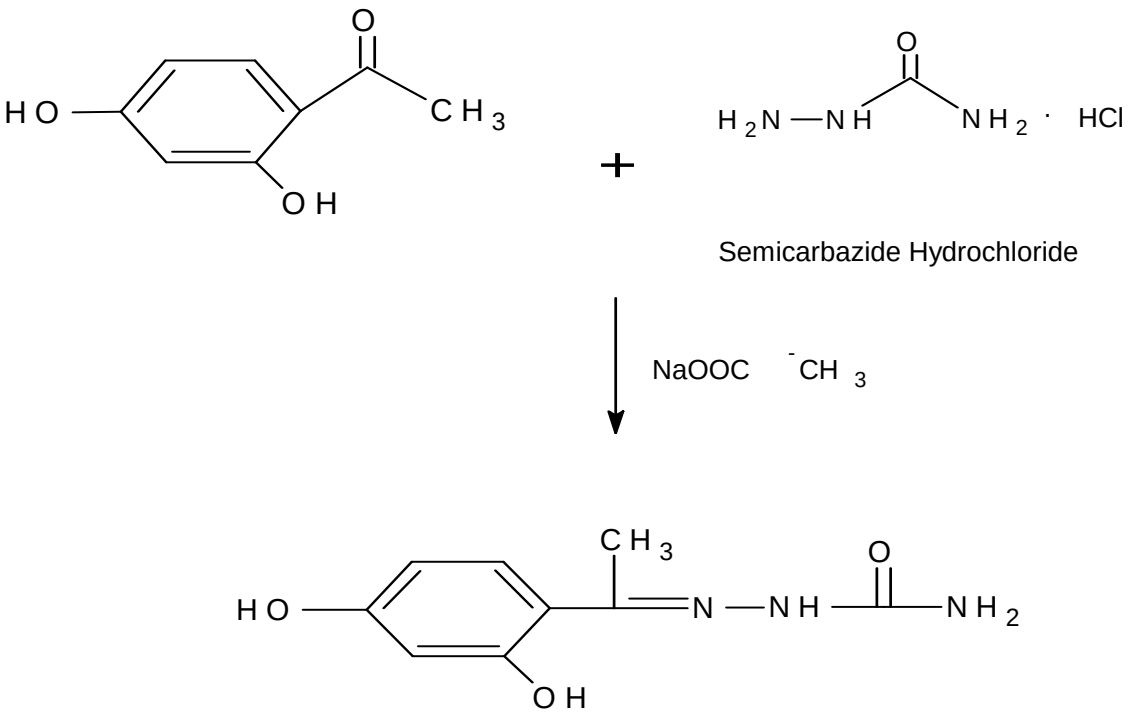

fig.3: Reaction scheme for the synthesis of nickel complex of of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

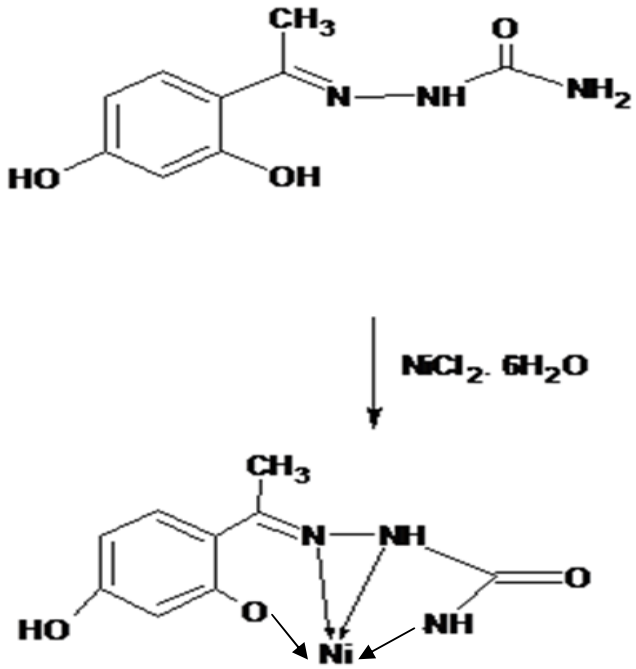

fig 4: IR for 2,4- dihydroxyacetophenone

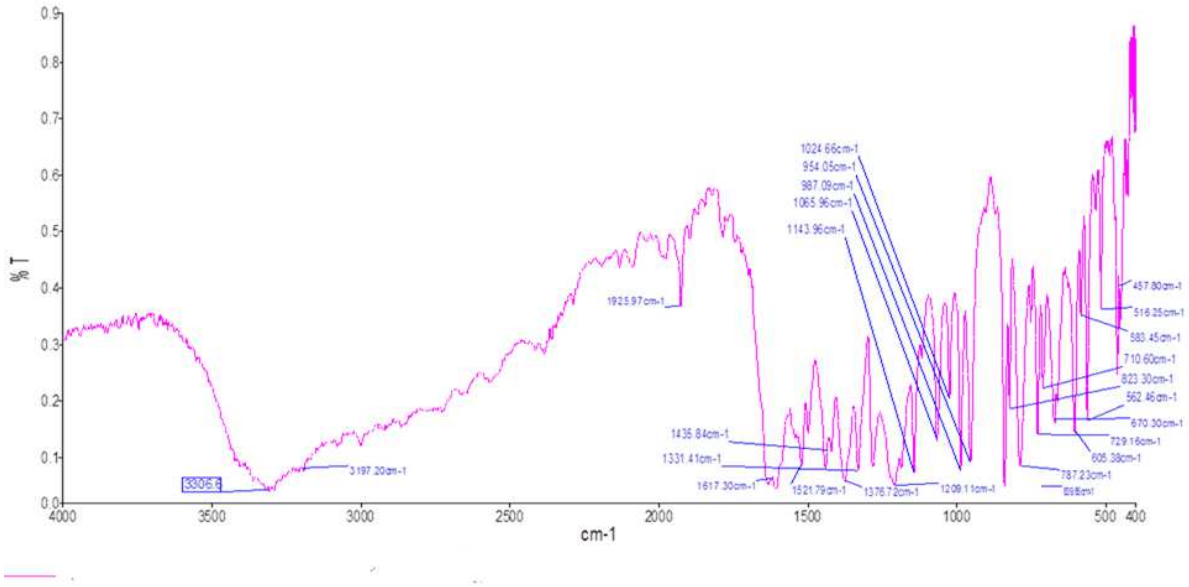

fig 5: IR for 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

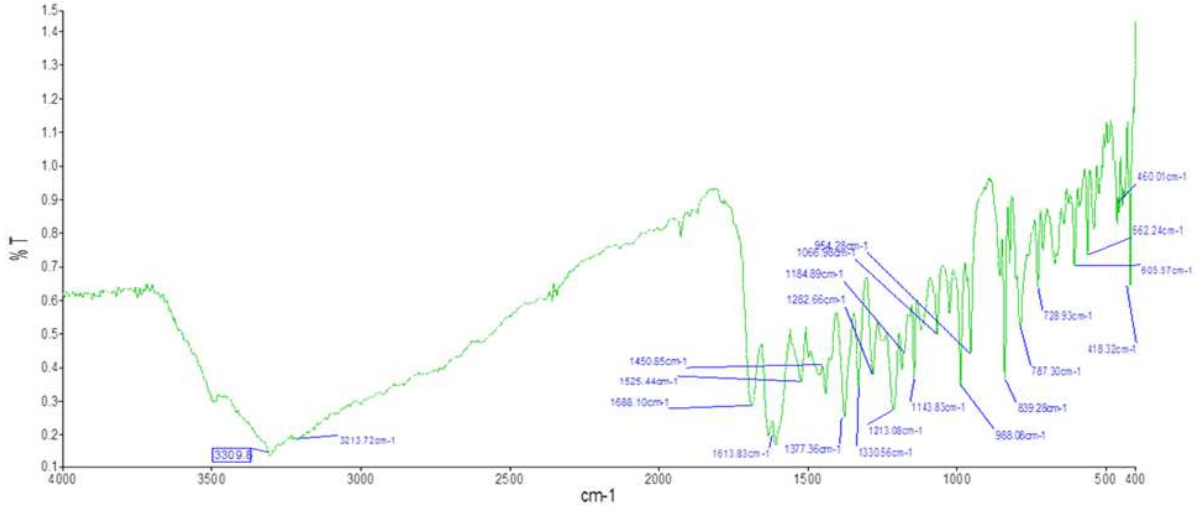

fig 6: IR for nickel complex of 2-[1-(2,4-dihydroxyphenyl)ethylene]hydrazinecarboxamide

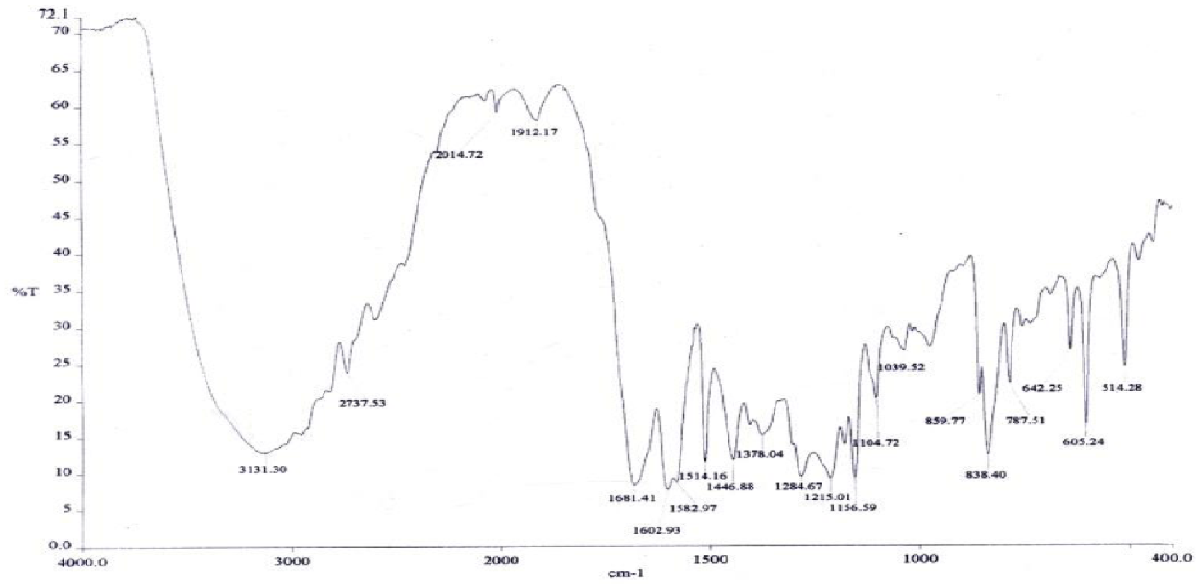

Table 1: physical and analytical data

Compound	color	M.P.(^{0}C)	λmax in nm	Elememtal analysis%		
				C	H	N
I	Off white	143	217	63.15	5.30	--
II	Pale yellow	201	342	59.01	3.90	5.45
III	Green	385	520	59.40	3.59	6.42

Table 2: spectral data

Group & vibration	**Frequency's in cm^{-1} I**	**Frequency's in cm^{-1} II**	**Frequency's in cm^{-1} III**
Aro, O-H Streching vibration	3307	3309	3131.30
Aro, C-H Streching vibration	3197.20	3212.72	2737.53
Aro, C=C Streching vibration	1521.79	1525.44	1514.16
OH parasubsituted	1925.97	1922	1912.17
OH orthosubsituted	1800	1805	-----
C=O conjugation	1617.30	1688.10	1681.41
Aro, C-O Streching vibration	1143.96	1143.83	1156.59
Para substitution	823.30	839.28	859.77
Ortho substitution	729.16	728.93	787.51
Aro, C-N streching	----	1213.08	1284.67
Tertiary amide	----	----	1602.93
M-O streching	----	----	514.28

Table 3: antibacterial activity

Entry	**Subs**	**Zone of Inhibition in mm**				
		Gram positive Bacteria			**Gram negative Bacteria**	
		M. luteus	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
1.	I	15	16	19	14	13
2.	II	15	17	20	16	14
3.	III	16	18	23	23	16
4.	Ampicillin	17	19	26	24	17

I 2,4- dihydroxyacetophenone

II 2,4- dihydroxyacetophenone Semicarbazone

III Ni complex of semicarbazone derivative of 2-[1(2,4dihydroxyphenyl)ethylene]hydrazine

Carboxamide