Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(11):14-19

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, purification, characterization and microbiological evaluation of herbo-bismuth complexes

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ABSTRACT

Metals are essential cellular components selected by nature to function in several indispensable biochemical processes for living organisms and possess good antibacterial activity. For present study we selected an herbal origin drugs such as Gallic acid, Quercetin, Catechin. In this work, we had synthesized the complexes of selected drugs with bismuth using its two different salts such as bismuth nitrate and bismuth citrate at neutral ph. The method of synthesis was further standardized in order to optimize the yields of title compounds, by altering one parameter at a time and keeping others constant. Purification of title compounds were done by multiple washing with solvents such as water, ethanol. The title compounds were characterized by Uv visible spectroscopy, IR spectroscopy, Elemental analysis, Inductively coupled plasma atomic emission spectroscopy (ICP-AES), Differential scanning spectroscopy and XRD analysis. Molar absorptivity ratio was determined, which indicates 1:1 formation of drug and metal complex. Karl Fischer analysis for synthesized complexes showed smaller percentage of water which indicated the fact of presence of coordinated water molecule with active molecule. To evaluate the changes in microbiological activity of parent drug after complexation, antibacterial studies were carried out by observing the changes in MIC (minimum inhibitory concentration) of the complexes and compared with that of parent drug. Our investigations reveal that formation of complexes results in increase in antibacterial activity of parent drugs and MIC values are decreased.

Keywords: Metals, Herbal Origin Drugs, Bismuth, Standardization, Purification, IR, DSC, XRD, ICP-AES, Molar Absorptivity, Karl Fischer Analysis, Microbiological Activity, MIC.

INTRODUCTION

Nature has been a potential source of therapeutic agent for years. An impressive number of modern drugs have been derived from natural sources. Plants have an ability to synthesize antimicrobial substances, most of which are phenols or their oxygensubstitutedderivatives. In the view of the same, herbal drugs considered for present study are – Gallic acid, Quercetin and Catechin. Gallic acid is atrihydroxybenzoic acid, a type of phenolic acid, found in gallnuts, sumac, witch hazel, tea leaves and seems to haveantimicrobial activity. Quercetin&Catechin are a plant-derived flavonoid possessesantimicrobial property. Modification of these existing drugs by complexing with metal atom or ion may result in better activity than parent drug. The ligand environment of transition metals (present in very low concentration *in vivo*) can be considerably altered upon the administration of a therapeutically effective. This change in balance between the metal ion and the ligand may have a profound effect upon the activity of drug against potentially susceptible bacteria. It has also been reported that the transport of organic ligands into the cells can be facilitated by the formation of metal complexes (Gao et al, 1995). Bismuth is known to possess good

antibacterial activity (Clitherow, 1991; Peter and Colm, 2005). Ranitidine-bismuth citrate complex is clinically available. Transport ofquinolones across the bacterial cytoplasmic membrane is strongly pH dependent, peaking at neutral pH(Furet et al., 1992). It has been proposed that zwitterionic and uncharged quinolone species are responsible for diffusion through cytoplasmic membranes (Kawai and Matsubayashi,1996) and the presence of metal ions result in a higher uptake of quinolones by bacterial cells compared to that of the drug alone (Ma et al., 1997). Therefore, the formation of metal complexes may increase the bioavailability of the metal ion or the ligand or both. It is believed that bismuth in complexed form is more stable and better tolerated (Clitherow, 1991). Hence, it was planned to synthesize the Herbo-bismuth complexes and to evaluate the same for their antimicrobial spectrum.

EXPERIMENTAL SECTION

Materials:
Selected herbal drugs i.e. Gallic acid; Quercetin and Catechin were procured Sigma Aldrich Corporation Ltd. Bismuth nitrate, Citric acid, Dimethyl Sulfoxide (DMSO) and Ammonia solution were also obtained from Sigma Aldrich Corporation.

Preparation of Herbo-Bismuth complxes (HBC) :
The title compounds were synthesized by following scheme:

Step 1) Preparation of bismuth citrate
Bismuth citrate was prepared by adding bismuth nitrate (5.74 g, 0.02 mole) to a solution of citric acid (8.40g, 0.043mole) in water (80ml). This mixture was heated on steam bath withfrequentstirring for 30 min. The precipitate obtained was washed with water and dried under vacuum to give bismuth citrate yield: 6.20g ,81%, m.p.-320-330°C (Reported 330°C Aldrich catalogue,2001,6.20 g, 76% M.P-310).

Step 2) Preparation of Drug-Metal complex.
The selected drugs (1 mmol) and bismuth citrate/ bismuth nitrate (1 mmol) were dissolved separately in 10 ml 0.1N HCl and mixed together in their respective reaction vessels. The reaction mixtures were stirred at room temperature with simultaneous adjustment of pH 7 with ammonia solution. After attaining desired pH, mixtures were refluxed for 6 to 10 hours with simultaneous checking and adjusting pH at7. They were then allowed to stand overnight. The precipitated complexes were filtered off, washed with ethanol, ammonia solution, water and finally dried in oven at 60°C.

Characterization of Herbo-Bismuth complexes (HBC):
Infra-red spectra of title compounds were recorded in the range of 4000-400 cm-1 with Schimadzu FT IR8300, using KBR pellet method. Ultraviolet (Uv) spectra wereefficiently recorded on Schimadzu 1700. Elemental analysis was performed using thermofinnagane-EA112 series NCHS Analyser. Bismuth content was determined using Thermo S- series atomic absorption spectrometer. Karl fisher titrimeter (Aqua Cal) was used for the determination of water content. DSC analysis was performed using MettlerToledo 821e, DSC (Mettler Toledo, Switzerland). The sample size used for DSC was about 5 mg. The scanning speed was kept at 10 C/min. X-ray Diffraction studies of synthesized complexes were carried out for Solid state characterization at Tata Institute of Fundamental Research by using X-pert diffractometer.

Karl –Fisher titrimetry for water content
Karl – Fisher titration was performed in order to find out the number of bonded water molecules. Karl – Fisher reagent was calibrated with disodium tartarate. An accurately weighed amount of compound was added to the dry methanol in KF reaction vessel and titrated against Karl – Fisher reagents. Titration was performed in triplicate to get reproducible results.

1)DEGRADATION POINT AND PERCENTAGE YIELD OF TITLECOMPOUNDS :

Table no-1

SR.NO	TITLE COMPOUNDS	MELTING POINTS (DECOMPOSITION) Onset of Decomposition.	PERCENTAGE YIELDS %
1	Gallic acid-bi complex(a)	330^0C	72.62
2	Quercetin – bi complex (b)	280^0C	67.56
3	Catechin – bi complex(c)	250^0C	70.62
4	Gallic acid-bi complex(a1)	330^0C	69.62
5	Quercetin – bi complex(b1)	280^0C	67.23
6	Catechin – bi complex (c1)	250^0C	68.32

(a,b,c indicates drug metal complex synthesized from bismuth citrate while a1,b1,c1 indicates drug metal complex synthesized from bismuth nitrate)

2)UV Spectral analysis

Ultraviolet (Uv) spectra were efficiently recorded on JASCO V-550 Uv/Vis spectrophotometer.

Table no-2

SR.NO	TITLE COMPOUNDS	λMAX(nm)	SOLVENTS USED
1	**Gallic acid-bi complex (a)** **Gallic acid-bi complex (a1)**	**215,268** **215,268**	**0.1N NaoH**
2	**Quercetin-bi complex (b)** **Quercetin-bi complex (b1)**	**220,315** **220,315**	
3	**Catechin-bi complex (c)** **Catechin-bi complex (c1)**	**268** **268**	

3)ELEMENTAL ANALYSIS

Elemental analysis was carried out using Thermofinnagane-EA112 series NCHS Analyser.

Theoretically calculated and practically observed percentage of Carbon, Hydrogen in title compounds are as follow:

Table no-3

COMPOUNDS		Gallic acid-bi Complex(a)	Catechin-bi complex (b)	Quercetin-bi complex (c)	Gallic acid-bi complex(a1)	Catechin-bi complex(b1)	Quercetin-bi complex(c1)
Empirical formula		C7H5O5.Bi H2O	C15H12O6Bi. H2O	C15H10`O7Bi. H2O	C7H5O5.Bi. H2O	C15H12O6Bi. H2O	C15H12O7Bi. H2O
% C	TC	21.232	34.935	34.190	21.232	34.935	34.190
	PO	22.781	34.112	33.698	23.781	32.959	35.094
% H	TC	1.783	2.742	1.913	1.783	2.742	1.913
	PO	1.564	2.678	1.757	1.695	2.670	1.832

TC- Theoretically Calculated , PO- Practically Observed

4)Inductively coupled plasma atomic emission spectroscopy(ICP-AES),

Table no: 4

SR.NO	Code of Samples Given	Name of Compound	Concentration of Bismuth (ppm)	% of Bismuth	
				Theoretically Calculated	Practically Observed
1	A	Gallic acid-bi complex (a)	678.9	52	50.3
2	B	Quercetin-bi complex (b)	560.06	40	42
3	C	Catechin –bi complex (c)	537.118	40.9	39.7
4	D	Gallic acid-bi complex (a1)	662.3	52	50.2
5	E	Quercetin -bi complex (b1)	551.17	40	41.2
6	F	Catechin –bi complex (c1)	550.25	40.9	41.2

Molar Absorptivity determination:
To find out the number of moles of ligand (drug) attached to the central metal atom (bismuth), molar absorptivity of the compounds of the compound was determined. Accurately weighed amount of compound was dissolved in suitable respective solvent and absorbance was recorded on a UV spectrophotometer at its λmax.Molarabsorptivityas obtained for the synthesized compound was compared with the molar absorptivity of the ligand to get the mole ratio of metal**:** ligand, which was found to be 1:0.93 for gallicacid bi complex,1:1.1 for quercetin bi complex and for 1:1.096 for catechin bi complex , thus it shows ratio of metal: ligand almost equal to 1:1. Molar

Absorptivity,Where A is absorbance and W represents weight of sample in milligrams.W = 5 mg, and Dilution factor = 100, in our experiment, we have kept weight and dilution factor as constant.

Evaluation of antibacterial activity:
The minimum inhibitory concentrations (MIC) of parent herbal drugs as well as synthesized complexes were determined by agar diffusion method using Brain Heart Infusion agar for antibacterial and sabouraud agar medium was used for antifungal studies as described by the National Committee for Clinical Laboratory Standards in its guidelines (NCCL Guidelines, 1993; United States Pharmacopoeia, 2002). Samples were initially dissolved in 0.1 N NaOH at concentration-75µl, 50µl,25 µl,10 µl. Isolates were grown for 24 h in nutrient broth to provide a turbidity of approximately 109 cfu/ml. Bacterial suspensions were diluted with soft agar containing tubes at 45–50 ◦C. These soft agar tubes were then poured over the Muller Hinton agar plates previously prepared and allowed to solidify under laminar flow for 15 min. Filter paper discs, previously sterilized were, placed over the agar plates containing bacterial suspensions. Sterile pipettes (0.2 ml) were used in aseptic conditions to add the compounds on filter paper disc. Sample solution (0.15 ml) was added to each disc. Same volume of the control

(DMSO) was also added on one of the other disc in each plate. The plates were then placed in an incubator at 37 ◦C within 15 min of addition of the compounds on the filter paper disc. After 18–24 h of incubation, the plates were examined and the diameter of zones of complete inhibition was measured by zone diameter measuring scale.

RESULTS AND DISCUSSION

Uv spectral analysis of all synthesized complexes such as Gallic acid-bi complex showed at λmax at 286 nm, Quercetin-Bi complexes at 315nm and Catechin-Bi complexes at 268nm, thus synthesized complexes showed a hypsochromic shift as compared to parent drug which indiacated that an auxochrome is no longer free to give absorbance at specified wavelength.

IR spectral data showed prominent absorption peaks for peculiar functional groups at the expected frequencies for all title compounds.IR spectrum of Gallic acid-bi complex does not showed any peak in range of 1690-1710cm-1 indicating disappearance of carboxyl group of gallic acid due to deprotonation of COOH group whereas IR spectra of Quercetin-bi complex and Catechin-bi complex showed broad peak at 3200cm-1indicating, there is shift in peak of hydroxyl group, which also suggested that hydroxyl groups are involved in complex formation. Carbonyl group at 1650cm-1 in quercetinremains as it is in the spectrum of its synthesised complex indicated that carbonyl group has not been involved in complex formation with metal.

Molar absorptivity determination has been carried out for synthesised complexes and parent drugs, which indicate that there is no much difference in molar absorptivity of parent drug and complex, Thus one mole of drug has been attached with one mole of metal i.e 1:1 formation of drug metal complex. Karl Fischer analysis for synthesised complexes showed smaller percentage of water (Gallic acid-bi complexes- 4.2%, Quercetin-bi complex-3.5% Catechin-bi complex-3.2%) which indicated the fact of presence of coordinated water molecule with active molecule.

CONCLUSION

So, present study involves synthesis of Herbo-bismuth complexes followed by its characterizational studies like UV, IR, DSC, atomic absorption spectroscopy, Karl- Fischer titrimetric analysis and elemental analysis. The complex was found to possess metal to ligand ratio of 1:1. It has been observed that complexation between bismuth ions and selected drugs takes place at pH 7. Agar diffusion method was used for antibacterial activity. Herbobismuth complexes (HBC) were found to possess better activity (lesser MIC value) than that of parent drug as wellas bismuth citrate. It was concluded that HBC can be a better an antibacterial agent.

Acknowledgements
The authors are thankful to SAIF,IIT Mumbai India for providing atomic absorption spectroscopy facility.TIFR,Mumbai for providing facility for X-ray diffraction studies.

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