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Characterisation of *phyllanthus amarus* herb by inductively coupled plasma mass spectrometric (ICP-MS) Analysis, Optical absorption and electron paramagnetic resonance (EPR) spectroscopic methods

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Abstract

A powdered sample of *phyllanthus amarus* herb of Kadapa district of Andhra Pradesh, India is used in the present study. ICP-MS analysis indicates that copper is present in higher concentration when compared to other elements. Through the Pb is toxic which is less than the permissible value. The evaluated soil and herb physico-chemical parameters are indicating that the sample is acidic in nature in comparison with the soil. An EPR study on powdered sample confirms the presence of Fe(III), Mn(II) and Cu(II). Optical absorption spectrum indicates that Fe(III) impurity is present in octahedral structure whereas Cu(II) is present in rhombically distorted octahedral environment. MIR results are due to carbonate fundamentals.

Introduction

Use of indigenous drugs of plant origin forms a major part of complementary and traditional medicine. Global demand for medicinal plant products such as pharmaceuticals, phyto-chemicals, nutraceuticals, cosmetics and other products is on the increase [1]. Herbal medicinal products may vary in composition and properties unlike conventional pharmaceutical products. Correct identification and quality assurance of the starting material is therefore an essential prerequisite to ensure reproducible quality of herbal medicine which contributes to its safety and efficiency [2]. Transitional metal ions play a major role in medicine [3].

However, macroscopic identification of medicinal plants is based on parameters like shape, size, colour, texture, surface characteristics, fracture characteristics, odour, taste and such organoleptic properties that are compared to a standard reference material. Microscopy involves inspection of broken as well as powdered and crude botanical materials. Chemical profiling establishes a characteristic chemical pattern to a plant material and its fractions of extracts. High performance Chromatography is routinely used as valuable tool for qualitative determination of small amounts of impurities. Many analytical techniques such as volumetric analysis, column chromatography, liquid chromatography and spectrophotometric methods are

frequently employed for quality control and standardization [4]. In order to elucidate the composition, structural properties of trace metal ions present in medicinal plants several techniques such as carbon-hydrogen-nitrogen-sulphur (CHNS), inductively coupled plasma mass spectrometric (ICP-MS) analysis, electron paramagnetic resonance (EPR) and optical absorption studies are employed in the present investigation. The common transition metal ion that occurs in many herbs is iron. *Phyllanthus amarus* is locally termed as *nela oosirika* in Telugu. The Spanish name of the herb is *chanca piedra*, means stone breaker or shatter stone. In Brazil, the herb is known as *qubra-pedra* or *arranca-pedras*. This herb has many biological activities and the benefits are attributed to many different chemicals. This herb used in the treatment of jaundice and also for treatment of kidney stones etc., [5]. In the present study, the authors determined the content of carbon, nitrogen and hydrogen using CHNS scan, the transition metal ions present, their valance state and site symmetries using ICP-MS analysis, EPR and optical absorption spectroscopic techniques.

Experimental

A *Phyllanthus amarus* herb originated from Pakkhirupalli of Kada Mandal and District of Andhra Pradesh, India is used in the present work. To know the organic composition of the compound CHNS scan has been carried. The results of the analysis indicate that carbon, nitrogen and hydrogen are present to the extent of 43.74 wt%, 2.06 wt% and 5.42 wt% respectively. Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Perkin Elmer Élan DRCII ICP-MS Toronto, Ontario, Canada) was used for the determination of trace elements present in the sample. The methodology of preparation of the sample, internal standards and optimization procedure already available in the literature is used [6] in the present investigations. The sample was also converted into ash using silica crucible by heating at 300 °C for two days in an electrical furnace. Sample yielded 70 grams of ash white in colour per kilogram of herb sample. Where as the soil contains 4% of organic matter. For the soil and the sample physico-chemical parameters are evaluated at room temperature. They are pH of soil is 7.58 and for herb sample pH is 5.68 . Using the formula [7]

$$Eh(mV) = E^{\circ} - \frac{59}{n} \log \frac{(Red)}{(Ox)} - \frac{59m}{n} pH$$

Here m/n ratio of protons to electrons.

E° Standard half-reaction reduction potential.

Thus the calculated value of Eh for soil is -230 mV and for herb sample is - 174 mV. EPR spectra of the sample in powder form are recorded at room temperature (RT) on a JEOL JES-TE100 ESR spectrometer operating at X-band frequencies ($\nu = 9.42259$ GHz), having a 100 KHz field modulation to obtain a first derivative EPR spectrum. Optical absorption spectrum of the compound is recorded at RT on a Carey 5E UV Vis-NIR spectrophotometer in mull form in the range 200-2500 nm.

Band component analysis was undertaken using the Jandel "PEAKFIT" software package which enabled the type of fitting function to be selected and specific parameters to be fixed or varied accordingly. Band fitting was carried out using a Gauss-Lorentz cross product function with the minimum number of component bands used for the fitting process. The Gauss-Lorentz ratio was maintained at values greater than 0.7 and fitting was undertaken until reproducible results were obtained with squared correlations of r^2 greater than 0.995.

Results and analysis

ICP-MS Analysis

SY-2, a syenite rock reference material was dissolved in the way as the sample in ICP-MS analysis as a calibration standard. This standard sample is well characterized and is a certified sample for most of the trace elements including rare earth elements. A full mass scan {mass to atomic number ratio (m/z) 45 – 239} is carried is used for quantification. A responsive curve (signal intensity vs mass to charge ratio) is obtained using a multi element standard solution for calibration of the ICP-MS. The data presented on a reference sample along with the sample is given in Table 1. The elemental analysis of the compound indicates that small amounts of transition metals are present in it. Copper is found in higher concentrations than other transition metals.

EPR Results

A *phyllanthus amarus* sample is made into a fine powder and transferred into a quartz tube for EPR measurements. The EPR spectrum of the sample recorded at room temperature is given in Fig. 1. For the sake of convenience, the resonances are marked as A (single line), B (single line), C (six lines) and D (single sharp line). The g value of A, B and D are 2.933, 2.400 and 2.016 Gauss.

It is well known that iron is the most common impurity in herbs and exhibit a wide variety of resonances. The resonance at $g = 2.933$ and the broad line under C, can be considered to be arising from Fe(III) impurity [8]. Therefore the resonance at $g = 2.400$ can be assigned to Cu(II). The hyperfine line from either ^{63}Cu or ^{65}Cu could not be resolved. The sharp peak at D, having a g value of 2.016 can be ascribed to a radical such as CO_3^- or NO_3^{2-} . The absence of any super hyperfine structure in the radical suggests that the radical may be CO_3^- [9].

The sextet (marked by C) has a g value of 1.977 and a hyperfine-coupling constant of 9.17 mT. An expanded form of this is given in Fig. 2. These spin Hamiltonian parameters suggest that ion responsible for this resonance is Mn(II) [10]. The hyperfine splitting (HFS) constant A can be calculated from the position of the allowed HF line using the formula

$$H_m = H_0 - A m - (35 - 4m^2) \left(\frac{A^2}{8H_0} \right).$$

Here H_m is the magnetic field corresponding to $m \leftrightarrow m$ in HF line

H_0 is the resonance magnetic field.

m is the nuclear spin magnetic quantum number.

The value of A at room temperature is found to be 9.8 mT. It is agrees with the experimental value. Further the strength of the HFS depends on the matrix and is mainly determined by the electro negativity of the anion neighbours. The magnitude of the HFS constant, A provides a qualitative measure of the ionic nature of bonding between the Mn(II) ion and its ligands. Van Wieringen [11] empirically determined a positive correlation between A and the ionicity of the manganese-ligand bond. It is found that Mn(II) is ionic in nature.

Hence the EPR analysis of the sample indicates that the paramagnetic impurities present in the herb are Fe(III), Mn(II), Cu(II) and a free radical, most probably CO_3^- .

Optical absorption Results

Based on the elemental analysis and ESR results the optical absorption spectrum is analysed. Though the EPR spectrum indicates the presence of Cu(II), Fe(III) and Mn(II) the observed optical absorption is attributed to Cu(II) and Fe(III) in the sample. This is because the concentration of Mn(II) in the herb sample is less than the other ions.

Optical absorption spectrum of *phyllanthus amarus* herb recorded in the mull form at RT from 200 - 2500 nm is shown in Fig.3. The spectrum shows energies at 8481, 12886, 14880, 16640, 20000, 25000, 31950 and 40160 cm^{-1} in the UV-Vis region. For easy analysis of the spectrum, the bands are divided into two sets 8481, 12886, 14880, 16640 cm^{-1} as first set and 12886, 14880, 20000, 25000, 31950 and 40160 cm^{-1} as second set.

The electronic configuration of Cu(II) is $[\text{Ar}] 3d^9$. In an octahedral crystal field, the corresponding ground state electronic configuration is $t_{2g}^6 e_g^3$ which yields 2E_g term. The excited electronic configuration, $t_{2g}^5 e_g^4$ corresponds to ${}^2T_{2g}$ term. Thus only one single electron transition ${}^2E_g \rightarrow {}^2T_{2g}$ is expected in an octahedral crystal field. Normally, the ground 2E_g state is split due to Jahn-Teller effect and hence lowering of symmetry is expected for Cu(II) ion and this state splits into ${}^2B_{1g}(d_{x^2-y^2})$ and ${}^2A_{1g}(d_z^2)$ states in tetragonal symmetry and the excited term ${}^2T_{2g}$ also splits into ${}^2B_{2g}(d_{xy})$ and ${}^2E_g(d_{xz}, d_{yz})$ levels. In rhombic field, 2E_g ground state splits into ${}^2A_{1g}(d_{x^2-y^2})$ and ${}^2A_{2g}(d_z^2)$ whereas ${}^2T_{2g}$ splits into ${}^2B_{1g}(d_{xy})$, ${}^2B_{2g}(d_{xz})$ and ${}^2B_{3g}(d_{yz})$ states. Thus, three bands are expected for tetragonal (C_{4v}) symmetry and four bands are expected for rhombic (D_{2h}) symmetry [12].

The first set of bands at 8481, 12886, 14880, 16640 cm^{-1} in the UV-Vis region are assigned to Cu(II) in rhombic symmetry. The general ordering of the energy levels for rhombic symmetry is as follows [12]: ${}^2A_{1g}(d_{x^2-y^2}) < {}^2A_{2g}(d_z^2) < {}^2B_{1g}(d_{xy}) < {}^2B_{2g}(d_{xz}) < {}^2B_{3g}(d_{yz})$. Accordingly the optical absorption bands observed for Cu(II) in *phyllanthus amarus* herb at 16640, 14880, 12886, 8481 cm^{-1} are attributed to the transitions from ${}^2A_{1g}(d_{x^2-y^2})$ to ${}^2A_{2g}(d_z^2)$, ${}^2B_{1g}(d_{xy})$, ${}^2B_{2g}(d_{xz})$, ${}^2B_{3g}(d_{yz})$ respectively. These observations are in agreement with those reported earlier [13-16] and the bands accordingly are ascribed to Cu(II) in octahedral coordination with rhombic distortion (D_{2h}) symmetry. Comparison of energies of the bands with their assignments for Cu(II) in rhombic octahedral coordination with ground state ${}^2A_{1g}(d_{x^2-y^2})$ are presented in Table 2.

The electronic configuration of Fe(III) is $[\text{Ar}] 3d^5$ with a half filled d-shell having one unpaired electron in each of the orbital. Hence, ground state configuration is $t_{2g}^3 e_g^2$ and has only spin forbidden d-d transitions. These occur from the ground state ${}^6A_{1g}(S)$ to the excited states $T_{1g}(G)$, ${}^4T_{2g}(G)$, ${}^4A_{1g}(G)$, ${}^4E(G)$, ${}^2T_{1g}(D)$, ${}^4E_g(D)$ and ${}^4T_{1g}(P)$ states in regular octahedral geometry. The degeneracy of E and T states is lifted with lower symmetry. The ${}^4T_{1g}(G)$ and ${}^4T_{1g}(P)$ transitions usually occur at energies around 12500 and 34000 cm^{-1} [17,18].

The spectral features are similar to Fe(III) when present in other inorganic compounds [19-21]. Accordingly the band observed at 12886 cm^{-1} is assigned to the transition ${}^6A_{1g}(S) \rightarrow {}^4T_{1g}(G)$. The band at 16640 cm^{-1} in the second set is also assigned to ${}^4T_{2g}(G)$. The third at 25000 cm^{-1} is assigned to ${}^4E(G)$ transition. Using Tree's polarization term $\alpha = 90^\circ$ [22] the energy matrices of the d^5 configuration are solved for various B, C and Dq values. The evaluated parameters which gave good fit are $B = 600$, $C = 2500$, $Dq = 865$ cm^{-1} . A comparison is also made between the calculated and observed energies of the bands and these are presented in Table -3. The band observed at 40160 cm^{-1} might be a charge transfer metal to ligand.

MIR Studies

The MIR spectrum of *Phyllanthus amarus* herb sample is shown in Fig. 4. In the MIR region, several bands are observed in the sample. These bands are due to overtones and combination tones of carbonate fundamentals.

The carbonate ion has six normal modes of vibrations. They are the symmetric stretching mode (ν_1), which is IR inactive, the out of plane bending mode (ν_2), the asymmetric stretching mode (ν_3) and in plane bending mode (ν_4). ν_3 and ν_4 are doubly degenerate. The fundamental frequencies of carbonate ion are $\nu_1 = 1063$, $\nu_2 = 879$, $\nu_3 = 1415$ and $\nu_4 = 680 \text{ cm}^{-1}$ [23]. The bands observed at 6877, 6357, 5740 cm^{-1} are assigned to combination tones ($\nu_1 + \nu_2 + 3\nu_3 + \nu_4$), ($4\nu_3 + \nu_4$), ($\nu_1 + 3\nu_2 + \nu_3 + \nu_4$) of carbonate ion. Also the bands observed at 5146, 4721, 4312 cm^{-1} are assigned to combination and over tones ($\nu_2 + 3\nu_3$), ($3\nu_1 + \nu_2 + \nu_4$) and $3\nu_3$ of carbonate ion. These assignments are in accordance with the spectral features of other carbonates present in inorganic compounds [24]. These assignments agree well with the calculated value and are given in Table 4.

Conclusion:

Phyllanthus amarus herb used in the present study contains low concentration of ferric iron, manganese and copper. Pb being extremely toxic. The reported value is 50.638 ppt, which is less than permissible level [25]. The EPR studies indicate the presence of Fe(III) (g value 2.933), Cu(II) (g value 2.400) and Mn(II) (g value 1.977) and a free radical (g value 2.016). This indicates that iron is in octahedral structure. Optical absorption spectrum of the sample reveals characteristic features of Fe(III) in octahedral site in addition to Cu(II) which is in the center of a rhombically distorted octahedron. The MIR results are due to carbonate fundamentals. The results of optical and EPR investigations conclusively prove that the site symmetry of Fe(III) ion is in an octahedral site and Cu(II) is in distorted rhombic environment.

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Table 1
Trace element concentrations (ppm) in phyllanthus amarus herb obtained by ICP-MS in comparison to syenite

Element/ Analyte	Mass measured	Standard Syenite (SY-2) Conc. Mean (ppt)	Phyllanthus amarus Conc. Mean (ppt)
Sc	45	6.775	3.157
V	51	45.917	10.385
Cr	52	9.328	2.333
Co	59	6.210	2.470
Ni	60	9.562	13.482
Cu	63	5.774	21.551
Zn	64	238.185	367.298
Ga	71	27.467	0.256
Rb	85	211.668	13.963
Sr	88	267.221	157.390
Y	89	127.478	0.347
Zr	90	278.813	2.587
Nb	93	28.858	0.093
Cs	133	2.459	0.064
Ba	148	468.755	143.727
La	139	77.152	0.826
Ce	140	178.970	1.511
Pr	141	18.918	0.162
Nd	142	73.724	0.493
Sm	152	16.418	0.203
Eu	153	2.469	0.089
Gd	158	17.275	0.127
Tb	159	2.548	0.030
Dy	164	18.605	0.070
Ho	165	3.881	0.030
Er	166	12.641	0.050
Tm	169	2.161	0.028
Yb	174	17.305	0.051
Lu	175	2.814	0.024
Hf	180	8.121	0.069
Ta	181	2.003	0.014
Pb	208	90.609	50.638
Th	232	409.916	0.414
U	238	307.653	0.322

Table 2
Comparison of energies of the bands with their assignments for Cu(II) in rhombic octahedral coordination with ground state $^2A_{1g}(d_x^2-y^2)$

Sample	$^2A_{1g}(d_z^2)$		$^2B_{1g}(d_{xy})$		$^2B_{2g}(d_{xz})$		$^2B_{3g}(d_{yz})$		Reference
	cm ⁻¹	nm	cm ⁻¹	nm	cm ⁻¹	nm	cm ⁻¹	nm	
Antlerite Cu ₃ SO ₄ (OH) ₄	8475	1180	9435	1060	10990	910	16390	610	[10]
Turquoise CuAl ₆ (PO ₄)(OH) ₈ ·4H ₂ O			14970	668			18354	545	[11]
ZPPH (ZnKPO ₄) ₆ ·6H ₂ O	7750	1290	9613	1040	12117	825	13330	750	[12]
Atacamite Cu ₂ (OH) ₃ Cl	8049	1242	10296	971	11083	902	15380	650	[13]
Phyllanthus amarus herb	8481	1179	12886	776	14880	672	16640	601	Present work

Table 3
Band headed data with assignments for Fe(III) in *phyllanthus amarus* herb.
 Site I Dq= 865, B= 600 and C =2500 cm⁻¹

Transition from $^6A_{1g}$	Wave number (cm ⁻¹)	
	Observed	Calculated
$^4T_{1g}(G)$	12886	12867
$^4T_{2g}(G)$	16640	16617
$^4E(G)$	25000	23276
$^4A_{1g}(G)$	20000	20300
$^4A_{2g}(F)$	31950	31780
	40160	C T

Table 4
Band head assignments for CO_3^{2-} in *phyllanthus amarus* herb
at room temperature.

Observed energies		Calculated energies cm^{-1}	Assignments
nm	cm^{-1}		
1454	6877	6867	$\nu_1 + \nu_2 + 3\nu_3 + \nu_4$
1573	6357	6340	$4\nu_3 + \nu_4$
1742	5740	5795	$\nu_1 + 3\nu_2 + \nu_3 + \nu_4$
1943	5146	5124	$\nu_2 + 3\nu_3$
2118	4721	4748	$3\nu_1 + \nu_2 + \nu_4$
2329	4312	4245	$3\nu_3$
2486	4022	4037	$3\nu_2 + \nu_4$

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