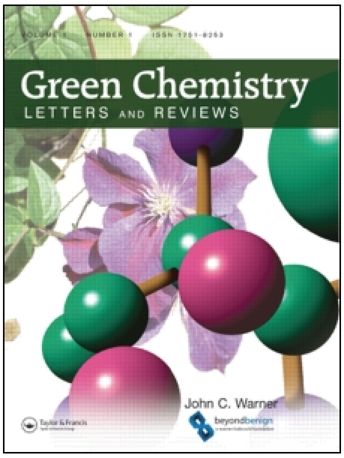

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RESEARCH ARTICLE

Extraction of humic acid from biological matrix – dry cow dung powder

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The increased local and global concern, for alarming environmental pollution, offers incentives to explore new green and clean materials and methods for safeguarding the environment. The generation of benign alternate routes for any step in chemical processes, is the need for today and tomorrow. In the present work, humic acid (HA) has been extracted from a green source, "dry cow dung powder", using simple, cost effective, and eco-friendly methods. HA has been extracted, isolated, and characterized by employing different spectroscopic methods. The process investigated herein imparts a boost to "Green Chemistry" a promising solution to many global environmental problems.

Keywords: humic acid; dry cow dung powder; green chemistry

Introduction

Humic acid (HA) is the fraction of humic substance (HS) which is insoluble in water under acidic condition ($pH < 2$) and is a polydisperse macromolecule comprising of amino acids, amino sugars, peptides and aliphatic compounds etc. HS are made up of HA, fulvic acid (FA), and other organic residues. Most of the data on HA, FA, and humins refers to the average properties and structure of a large assembly of components of diverse structure and molecular weight. HA is documented to interact with over 50 elements (*1*) from the Periodic Table, carcinogenic moieties, nutrients, radionuclides, toxic metals, and anthropogenic compounds.

Hence, it is employed in varied facets in veterinary medicine, in agriculture, industries, environment, and even in medicine. HA is known to play a vital role as a chemotherapeutic agent and exhibits anti-viral, anti-microbial, anti-inflammatory, and anti-coagulant properties (*2*). A derivative of HA has potent HIV-1 (*3*) and Herpes inhibiting properties.

The structure of HA is shown in Figure 1. It forms aggregates of elongated bundles of fibers at a low pH and an open and flexible structure perforated by voids at a high pH. The voids can trap and adsorb both organic and inorganic entities if the charges are complementary. This inherent property imparts to HA the characteristics of a natural adsorbent. HA sequesters anthropogenic organic compounds, photosensitizes chemical reactions, and complexes with heavy metals as well as with carcinogens.

HA originates mostly in the abiotic niche of the lithosphere and hydrosphere due to the humification of biodegradable entities. Humification is highly sensitive to pH, temperature, and climatic conditions. Hence, some degrees of differences are observed in the physical and structural properties of HA, if geographical origin differs. Deep lithosphere-based HA (*4*) (Lignite) contains low rank coal which is undesirable and performs poorly as a growth stimulator. Surface lithosphere-based HA (Leonardite) contains contaminants such as clay, shale, silica, gypsum, and other anthropogenic pollutants. Hydrosphere-based HA (*5*) (Marine or River) contains a very low percentage of HA, abundant undesirable micro flora, and water pollutants. In addition, the pre and post-process for the concentration of the raw material is time consuming and requires a higher degree of purification. This leads to the generation of many undesirable chemical steps, adding to the cost of the actual extraction process as well as questioning the statutory norms for environmental safety.

In the present work, we have achieved the green process for the extraction of HA, from the biological matrix of dry cow dung powder following the principles of green chemistry (*6*). The process is cost and energy efficient due to renewable and freely available feed stock, simple reagents used at room temperature and ambient pressure. In addition, a catalyst or auxiliary substance of any kind is not applied throughout the process. The dry cow dung powder is a bio-organic, polymorphic fecal residue of

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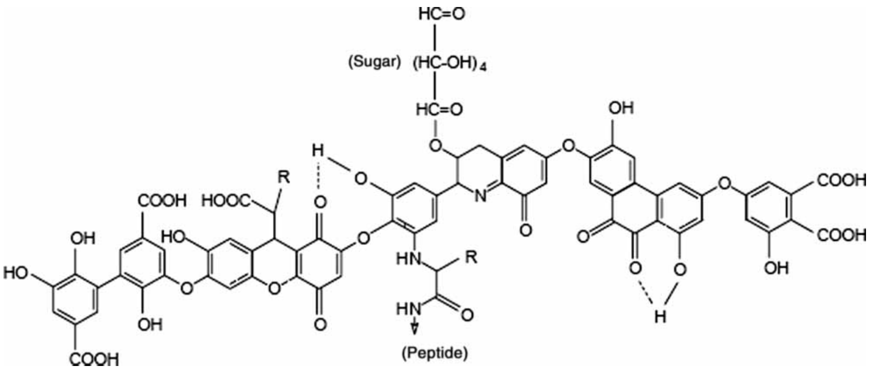

Figure 1. Model structure of humic acid according to Stevenson (*7*).

the bovine species. It is the digested residue of herbivorous matter which is acted upon by symbiotic bacteria residing within the animal's rumen. Hence, resultant product is enriched in minerals, carbohydrates, fats, proteins, enzymes, bile pigments, many aliphatic as well as aromatic species and "Humic acid". Being biodegradable, dry cow dung powder is non-hazardous. The dried form is the result of the humification process, enhancing the concentration of HA in it.

The process of purification (pre and post-concentration) of raw material in the present work is simpler and easier compared to those which are currently utilized by other synthetic sources. After separating HA from dry cow dung powder, the remaining residue left is enriched with humin, and other inorganic and organic complex substances that also have useful properties. Thus the entire process is easily carried out at the laboratory scale as the extraction process is very simple with no risk of accidents.

Materials and methods

All the chemicals used were of analytical grade. Dry cow dung (100 mesh) was provided by Keshav Shrushti, Research Centre, Mumbai. Due precautions were taken to avoid contaminations. Double distilled water was used for the conditioning of HA. Standard HA of LOBA Chemicals was used during experimental work. The extraction process was a batch type neutralization reaction at room temperature. The method of HA extraction from abiotic matrix, i.e. soil, peat, river, leonardite, etc. other than dry cow dung powder is given by International Humic Substance Society (IHSS). These methods employ various ion-exchanger resins, large number of chemicals, and sophisticated instruments (*8*). This leads to a lesser green extraction process in comparison with the simple extraction procedure devised in the present work.

Results and discussion

The FTIR spectrum of standard HA and extracted HA are shown in Figures 2 and 3 respectively. The description of absorption peaks is given in Table 1. According to the 3N–6N rule for non-linear molecules strong normal vibration bands are obtained. The standard HA shows vibration bands at 3704.2, 3632.1, 3421.0, 1648.1, 1438.9, 1114.2, 1049.3, 919.4, 804.0, 695.8, 551.5, and 479.4 cm^{-1}. Similarly, extracted HA shows vibration bands at 3422.8, 2932.3, 2860.1, 1720.2, 1648.1, 1525.5, 1460.5, 1114.2, 1042.1, 544.3, and 472.1 cm^{-1}. The FTIR spectra of both HA, i.e. standard and extracted, show an identical peak at 1114.2 cm^{-1} for the tertiary amine with aryl or alkyl substitutes and 1648.1cm^{-1} for the primary aliphatic amines. Shifts in absorption position may be caused by the factors such as intra and inter molecular hydrogen bonding, steric effect, physical state, degree of conjugation, and matrix effect. Both standard HA and extracted HA samples are different with respect to the matrix. The standard HA is from abiotic origin, whereas HA extracted from dry cow dung powder has biotic origin which gives rise to shifting in the position of bands. For the primary alcohols (C–O), a broad band is observed at 1040–1060 cm^{-1}. In the same region we observed band at 1049.3 and 1042.1 cm^{-1} for the standard HA and extracted HA, respectively.

At 3500–3400 cm^{-1}, broad band of primary amine (N–H) is normally observed. We obtained the band at 3421.0 and 3422.8 cm^{-1} in standard and extracted HA respectively. The band in the region of 551.5 and 544.3 cm^{-1} in the standard and extracted HA corresponds to chloroalkanes. The peculiar band at 1720.2 cm^{-1} in the extracted HA signifies the presence of lactose sugar and its derivatives which confirms its origin from lactating species, whereas the standard HA having an abiotic matrix does not show any band in this region. Raman spectra of standard

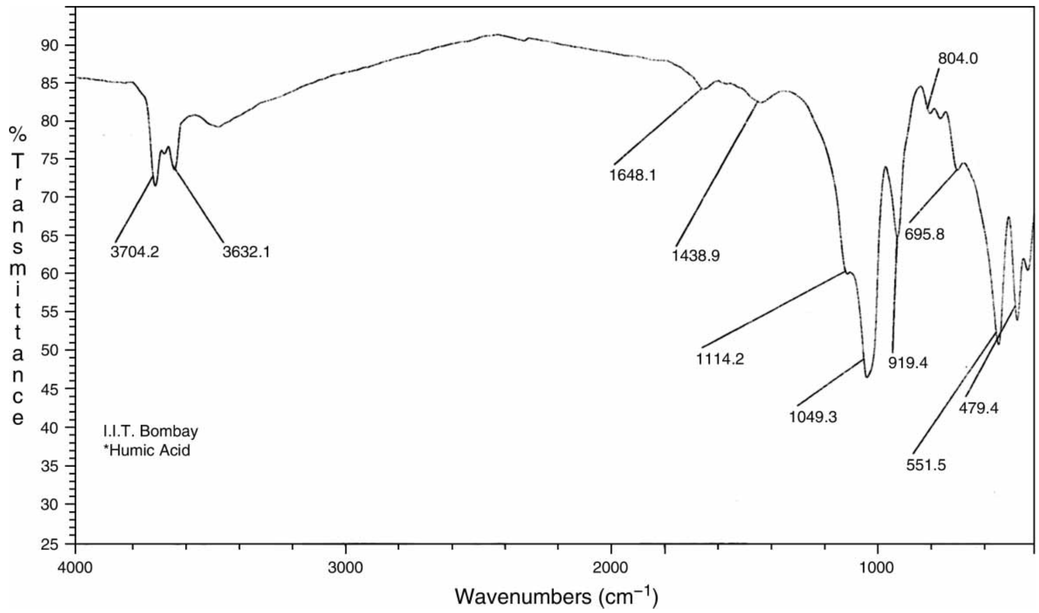

Figure 2. FTIR spectra of standard HA.

HA in Figure 4 and extracted HA in Figure 5, depict the absorption patterns. Description of absorption bands are given in Table 2. The broad band near the region of 1500–1600 cm^{-1} is due to absorption of (C$=$C) aromatic ring chain strong vibration at 1580, 1600 cm^{-1}, (C–NO$_2$) asymmetric medium vibration at 1530–1590 cm^{-1}, (N$=$N) aliphatic medium vibration at 1550–1580 cm^{-1}, and (X–Metal–O) strong vibration at 150–450 cm^{-1}. The spectra of standard HA shows a sharp narrow absorption band at 150–450 cm^{-1} indicating presence of heavy metals such as Iodine, Selenium, Silicon, etc. as stated in Table 2. A spectrum of extracted HA is devoid of an absorption band at that region which indicates the absence of any contamination of metal ion. This concludes that dry cow dung does not induce any undesirable matrix effect.

Experimental

For the extraction of HA from dry cow dung powder, the following methodology was used:

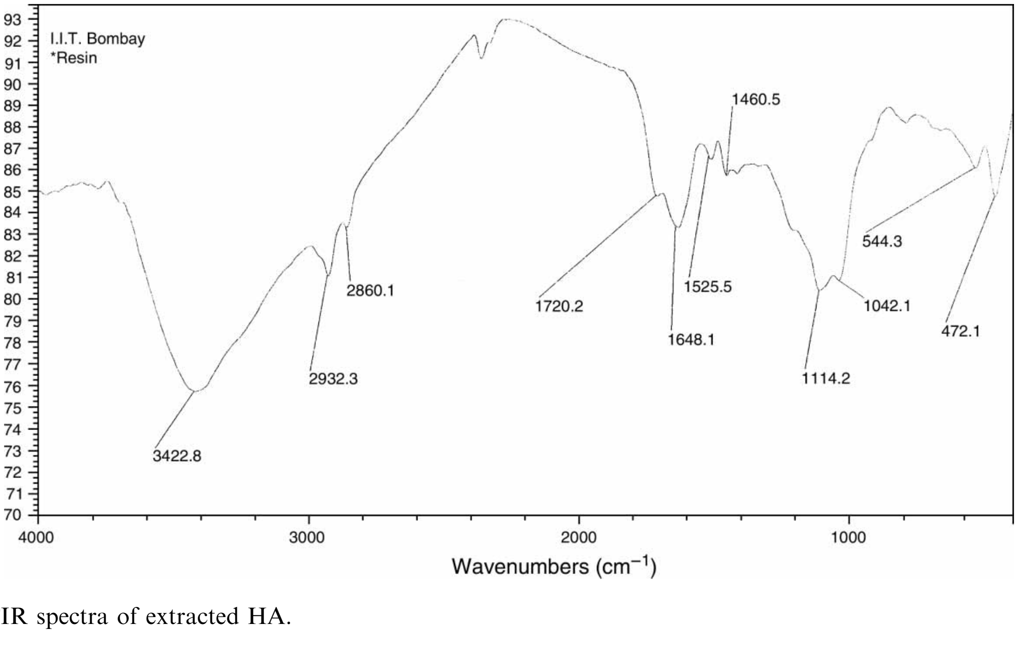

Figure 3. FTIR spectra of extracted HA.

Table 1. Interpretation of FTIR spectra.

No.	Functional group	Compounds	Wave number (cm^{-1})	Standard HA	Extracted HA
1.	C=O	Aldehydes, ketones, carboxylic acid, esters	1760–1670 (s)	–	1720.2
2.	N–H	Amines	1650–1580 (m)	1648.1	1648.1
3.	C–N	Carbon single bond	1340–1020 (m)	1114.2 1049.3	1114.2 1042.1
4.	C=O	Aromatic monomeric alcohols, phenols	3640–3160 (s)	3421.0	3422.8
5.	C–O	Saturated secondary or cyclic tertiary amine	1125–1085 (s)	–	–
6.	O–H	Carboxylic acid	3000–2500 (b)	–	2932.3 2860.1

1. Different series of alkali with range of 0.1–1.0 M of NaOH, KOH, Na_2CO_3, and $NaHCO_3$ were tested for the neutralization of dry cow dung powder. The best results were obtained with 0.1 M NaOH.
2. For the re-acidification process acids such as HCl, HNO_3, and H_2SO_4 in the molarity range of 0.1–1.0 M were tested. The best result was obtained with approximately 0.1 M HCl.
3. For 100 mL of neutralized supernatant 15 mL of ~0.1 M HCl is required.

Dry cow dung powder (100 mesh) was mixed with distilled water and stirred for 20 minutes at room temperature for the removal of hay and other light non-humic particles by the decantation process. After separation of the non-humic materials from the dry cow dung powder, the slurry thus obtained was stirred for 24 h with 0.1 M NaOH. The entire mixture was allowed to settle and then filtered. The filtrate contained solubles such as HA, FA, and other biological matter, whereas the residue contains humins, ulmic acid, and insoluble bio-organic matter. HA is insoluble at low pH, hence for the precipitation of HA the filtrate was acidified by slowly adding 0.1 M HCl at room temperature (pH $\approx$1) with constant stirring so as to minimize the heat of neutralization and it was centrifuged. The centrifugate contains other organic acids, along with FA which remains in solution after the removal of HA by acidification. The remaining HA thus obtained was thoroughly washed with double distilled water until a negative chloride test was achieved. The HA was then dried in an oven at 383 K. We have obtained 9–10% extraction of HA. Identification and characterization of HA were carried out using FTIR and Raman spectroscopy and compared with standard HA. FTIR spectra have been obtained on a Nicolet Instrument Corporation-USA, model-MAGNA 500 with specification range of 4000 cm^{-1}–50 cm^{-1}, from IIT Mumbai. Raman spectra have been obtained from Gemmological Institute of India, Mumbai, on a RENISHAW Laser Raman Spectrometer equipped with 325, 514.5, and 785 nm laser excitation and CCD detector with confocal microscope.

Conclusion

We have developed a simple, efficient, and eco-friendly method for the extraction of HA from dry cow dung powder. The presence of HA in the "Dry cow dung" (about 10%) has been investigated and is further confirmed by comparing and analyzing

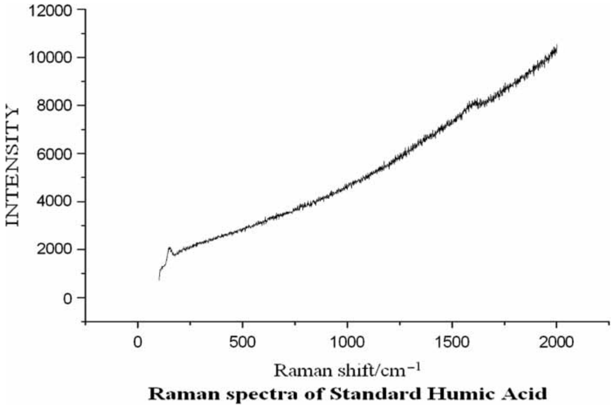

Figure 4. Raman spectra of standard HA.

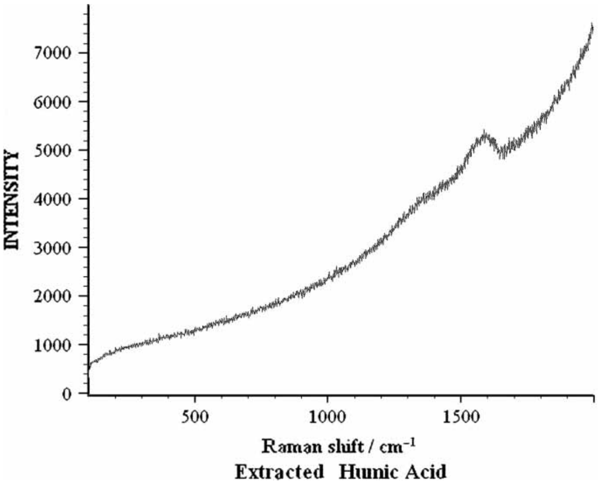

Figure 5. Raman spectra of extracted HA.

Table 2. Interpretation of Raman spectra.

No.	Functional group	Raman Shift/ (cm^{-1})	Band type
1.	$C–NO_2$	1530–1590	Asymmetrical medium
2.	N=N (Aliphatic)	1550–1580	Medium
3.	C=N	1610–1680	Medium
4.	C=C	1500–1900	Strong
5.	C=O	1680–1820	Medium
6.	X–Metal–O	150–450	Strong
7.	Se–Se	290–300	Strong
8.	C–I	480–660	Strong
9.	S–S	430–550	Strong
10.	Si–O–Si	450–550	Strong

spectral data with standard HA. This study confirms the efficiency and applicability of cow dung, to be used as a raw material for the extraction of HA. Hence developed methodology for the extraction of HA is a natural boon and will surely give impetus to Green Chemistry. From all these characteristics of dry cow dung powder, we can conclude that the methodology developed follows all 12 principles of Green Chemistry (*7*), hence it is a Green process.

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