Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

Naked-Eye Sensing of Phytic Acid at Sub-Nanomolar Level in 100% Water Medium by Charge Transfer Complex Derived from off-the-shelf Ingredients

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Materials and Methods

All reagents including starting materials, solvents and silica gel (for TLC and column chromatography) were obtained from the local commercial sources and were used without further purification. Solvents were distilled and dried prior to use (if necessary). FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). ^{1}H-NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 MHz. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane (TMS). Mass spectra were recorded on Micro mass Q-TOF Micro TM spectrometer.

UV-visible and fluorescence Experiment

The UV−vis and fluorescence spectra were recorded on a Shimadzu model 2100 UV-vis spectrometer and Cary Eclipse spectrofluorimeter respectively. In the emission experiments, the slit widths (for both the excitation and emission channel) was fixed at 5 nm and the excitation wavelength was chosen 400 nm. To monitored the effect of pH, sensing experiment were performed in buffered media of different pH (HCO_2Na/ HCl buffer for pH 2, Tris/HCl for pH 7 and $Na_2B_4O_7{\cdot}10H_2O$/NaOH for pH 12). For spectral studies with phytic acid, the solution of pyranine and methyl viologen was mixed in 1:1 ratio in water and incubated for 30 min at room temperature.

Time-resolved fluorescence spectroscopy

Fluorescence lifetime values were measured by using a time-correlated single photon counting fluorimeter (Horiba Jobin Yvon). The system was excited with nano LED of Horiba - Jobin Yvon with pulse duration of 1.2 ns. Average fluorescence lifetimes (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

$$\tau_{av} = (a_1\tau_1^2+a_2\tau_2^2 +a_3\tau_3^2)/(a_1\tau_1+a_2\tau_2+a_3\tau_3)$$

Where a1, a2 and a3 are the relative amplitudes and τ_1, τ_2, and τ_3 are the lifetime values, respectively. For data fitting, a DAS6 analysis software version 6.2 was used.

Real-Life Sample Analysis

The grain samples, used in present study, were collected from local markets and washed thoroughly with distilled water. Then ~0.5 g amount of fine-ground grains

(using mortar pestle) was extracted with 10 mL of 0.5M HCl for 2 h at room temperature. The suspension was centrifuged at 5000 rpm for 5 min and the supernatant was subsequently purified by anionic-exchange resin using a 0.7M NaCl solution as eluent. The solution containing PA was adjusted to neutral pH (by addition of NaOH) and then diluted with pH 7.4 buffer to a 200 mL volumetric flask. For spectral analysis the samples were further diluted (x 40) using the same buffer solution.

Determination of Zeta Potential

Zeta potential values were determined at room temperature using a Malvern Zetasizer Nano ZS particle sizer (Malvern Instruments Inc., Westborough, MA). Samples were prepared and examined under dust-free conditions.

Additional Spectral Data

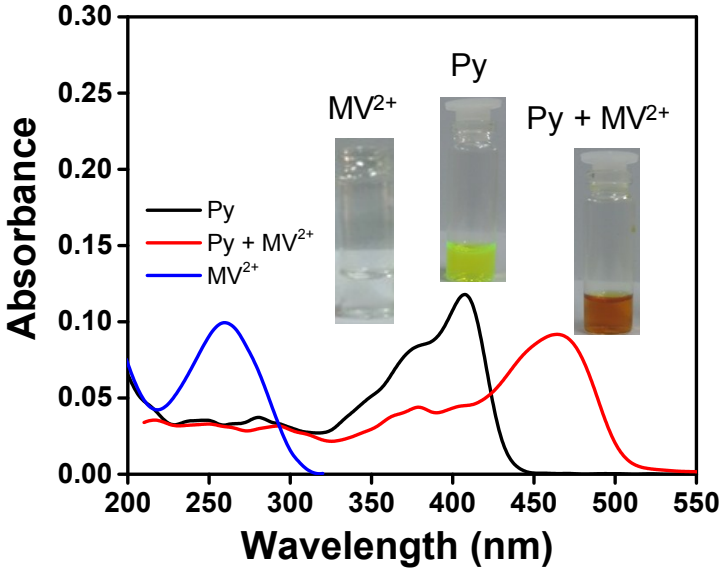

Figure S1. UV-Visible spectrum of pyranine (Py) with methyl viologen (MV^{2+}) in 1:1 ratio at pH 7.4 in PBS buffer.

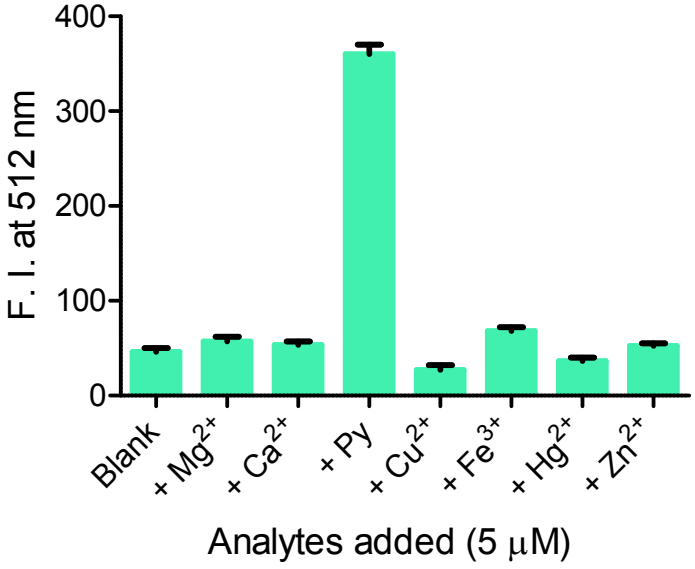

Figure S2. Change in fluorescence intensity of CT complex (λex = 400 nm, 15 μM) at 512 nm upon addition of different metal ions (5 μM) at pH 7.4 in PBS buffer.

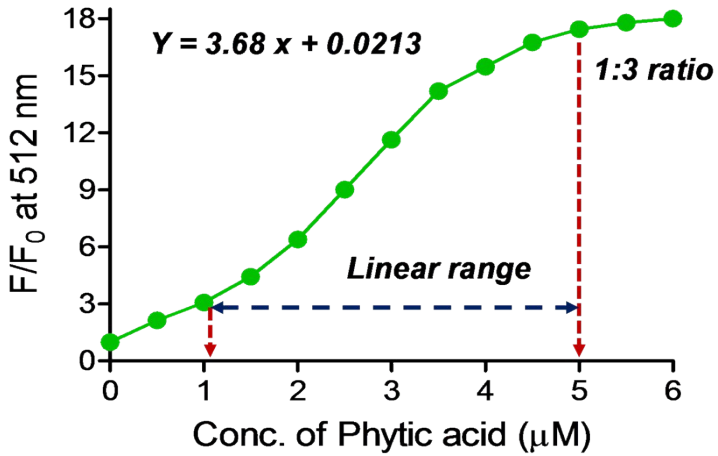

Figure S3. Change in fluorescence intensity of CT complex (λex = 400 nm, 15 μM) at 512 nm upon addition of phytic acid (0-6 μM) at pH 7.4 in PBS buffer.

System	Medium	Linear range	Detection limit	Application	Reference
PFBT-COOH Pdots/Fe^{3+}	pH 5.7 buffer	2.5 – 450 μM	0.63 μM	In corn samples	*Anal. Methods*, 2016,**8**, 7755-7761
Polyelectrolyte dots/Fe^{3+}	pH 5.9 buffer	0 – 8 μM	10 nM	In HeLa cells	Talanta, 2019, 202, 214–220
Tetra naphthoimidazolium receptor	pH 7.4 buffer	300 nM - 1 μM	0.23 μM	In HeLa cells	*Chem. Commun.,* 2014, 50, 5851--5853
GSH@AgNCs/Fe^{3+}	pH 5.0 buffer	4 – 20 μM	1.0 μM	In corn samples	*Anal. Methods*, 2016,**8**, 6382-6387
CdSe@SiO2-CDs/Fe^{3+}	water	0.08 - 1.6 mM	1.5 μM	In water samples	Chem. Eng. Trans., 2018, 70, 2065-2070
Carbon dots/ Fe^{3+}	pH 5.7 buffer	0.68–18.69 μM	0.36 μM	In corn samples	Biosens Bioelectron, 2015, 70, 232–238
Graphene QDs@GSH/ Fe^{3+}	pH 7.4 buffer	0.05 – 3 μM	14 nM	Corn samples/ blood serum	Anal. Chim. Acta, 2018,1039, 74-81
Pyranine + Methyl viologen	pH 7.4 buffer	0 – 5 μM	**0.56 nM**	Different grain samples, paper strips	Present work*

Table S1. A comparison of present method with other previously known optical probes.

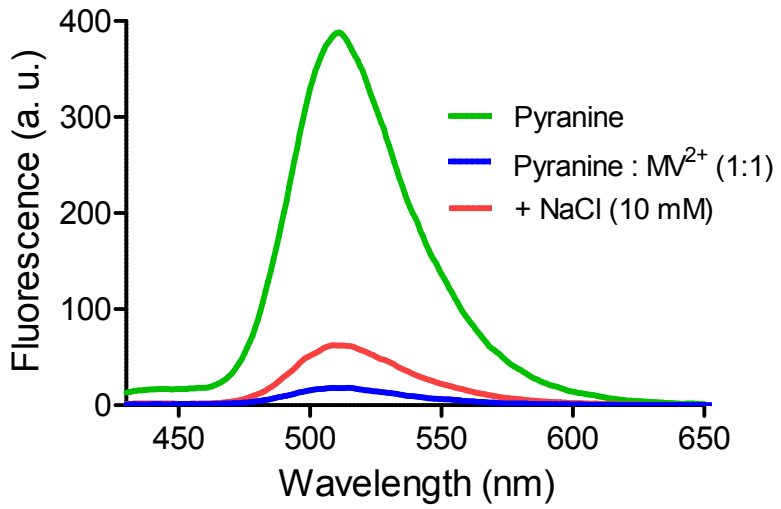

Figure S4. Fluorescence spectra of CT complex (λex = 400 nm, 15 μM) upon addition of NaCl (10 mM) at pH 7.4 in PBS buffer.

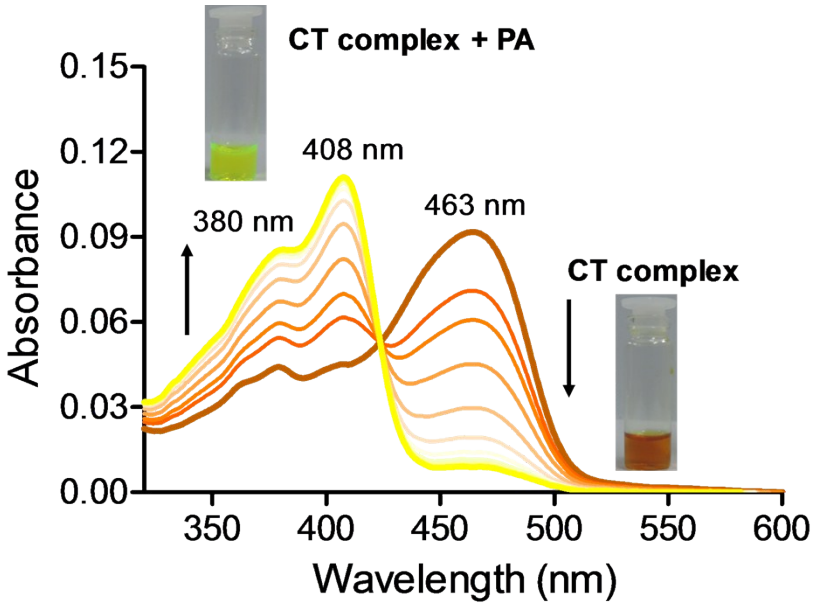

Figure S5. UV-Visible spectra of CT complex (15 μM) upon addition of phytic acid (0 – 8 μM) at pH 7.4 in PBS buffer.

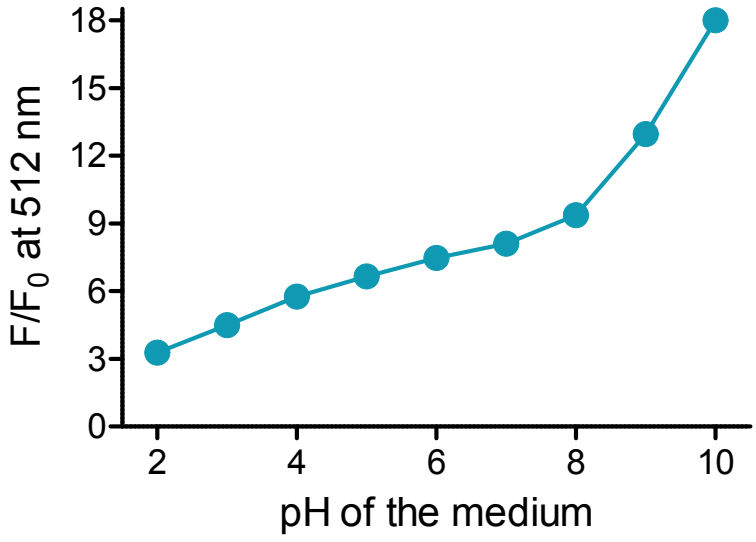

Figure S6. Change in fluorescence intensity of CT complex (λex = 400 nm, 15 μM) upon addition of phytic acid (0.5 equiv.) at different buffer medium.

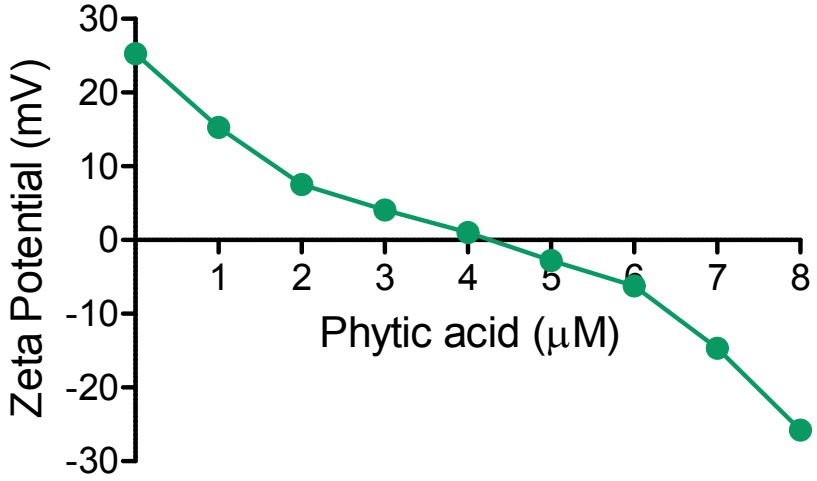

Figure S7. Change in zeta potential value of MV^{2+} (15 μM) upon addition of phytic acid (0 – 8 μM) at different buffer medium.

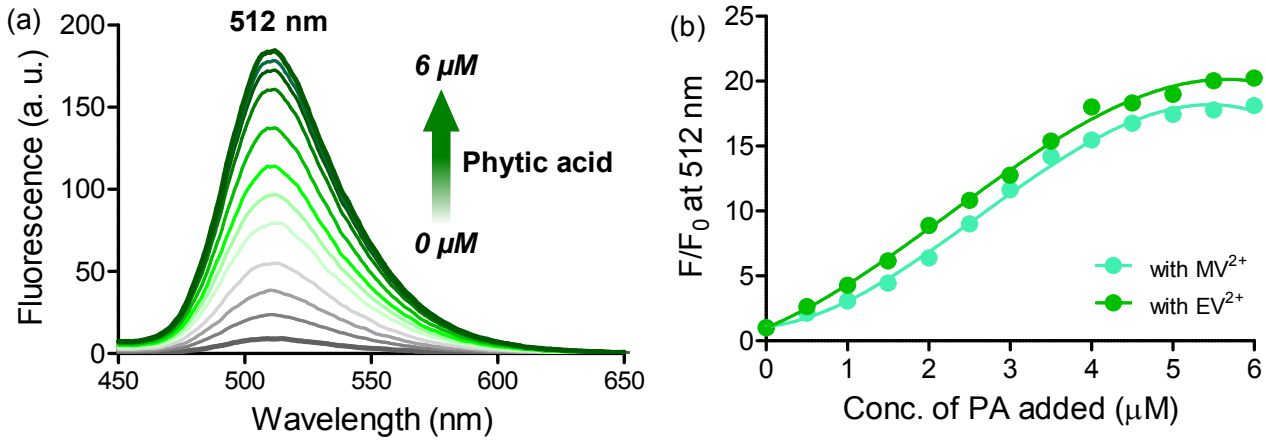

Figure S8. (a) Fluorescence titration of Py + EV^{2+} CT complex (15 μM, λex = 400 nm) with phytic acid (0-6 μM) in buffered medium (PBS, pH 7.4). (b) Change in fluorescence intensity of CT complexes (λex = 400 nm, 15 μM), made of pyranine with MV^{2+} and EV^{2+}, at 512 nm upon addition of phytic acid (0-6 μM) at pH 7.4 in PBS buffer.

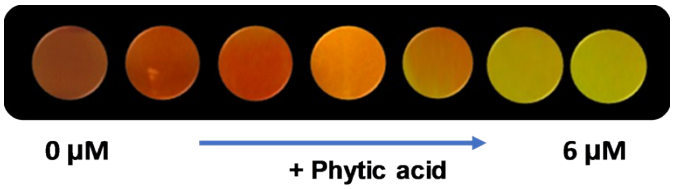

Figure S9. Change in color of the paper strips (CT-complex coated) upon addition of phytic acid.

Grain Samples	F/F_0 (at 512 nm)	Phytic acid calcd. (μM)	Phytic acid calcd. (g/100 g)	Phytic acid estimated* (g/100 g)	Error (%)	Phytic acid content (g/100 g)**
Mazie	3.65 ± 0.08	1.00	1.06	1.02	3.9	0.72 – 2.22
Wheat	1.85 ± 0.03	0.53	0.56	0.54	3.7	0.39 – 1.35
Oat	2.42 ± 0.05	0.68	0.72	0.75	4.0	0.06 – 1.08
Rice	1.08 ± 0.02	0.33	0.35	0.36	5.2	0.42 – 1.16

*calculated by commercially available phytase enzyme assay (K-PHYT).
**Phytic acid content reported in the literature

Table S2. Estimation of phytic acid (g/ 100 g) present in different grain samples using present method and commercially available K-PHYT assay.