Antioxidant and nematicidal properties were evaluated for \( R. \) emodi extractives which are extracted by standardizing and adopting accelerated solvent extraction (ASE) method along with traditional Soxhlet extraction. The extracted material was separated using flash chromatography and the separation conditions and solvents were standardized for the extracted plant constituents. The purity was detected by using analytical reverse phase high pressure liquid chromatography (HPLC). LC-MS/MS detection in the direct infusion mode of the isolated, purified products afforded four anthraquinones, characterized by their infrared spectra (IR) and \(^1\)H spectra as chrysophanol, physcion, emodin, and aloe-emodin. Five antraquinone glucoside derivatives and piceatannol-3-O-\( \beta \)-d-glucopyranoside have also been detected from the extracted product. During \textit{in vitro} evaluation the antioxidant potential of methanolic crude extract (CE1) was the highest, followed by ethyl acetate crude extract (CE2) and chloroform extract (CE3) in DPPH radical scavenging activity. The CE1 also demonstrated outstanding nematicidal activity as compared with other extracts, pure anthraquinones, and even positive control azadirachtin. The study conclusively demonstrated the antioxidant potential of \( R. \) emodi extracts and also its ability in extenuating the \textit{Meloidogyne incognita} (root-knot nematode). The bioassay results can be extrapolated to actual field condition and clinical studies.

1. Introduction

\textit{Rheum emodi} (family: Polygonaceae), commonly known as Indian or Himalayan rhubarb, is a perennial herb and a potential source of bioactive molecules against pests and diseases. It is distributed in the temperate and subtropical alpine region of the Himalayas. Ethnomedical uses of \textit{Rheum emodi} have been recorded from India, China, Nepal, and Pakistan for 57 different types of ailments [1, 2]. \textit{Rheum emodi} contains different secondary metabolites that are categorized as anthraquinones, stilbenes, anthrones, chromones, oxantrone ethers and esters, flavonoids, carbohydrates, lignans, phenols, and sterols. The major constituents of this herb are hydroxyanthraquinones and their glycosides which are responsible for various physiological activities. Some of the bioactive compounds identified in these plants extracts include rhein, emodin, aloe-emodin, chrysophanol, physcion, and their glycosides [3]. Other complex compounds have also been identified as torachrysone-8-O-\( \beta \)-d-glucopyranoside, sulphated emodin glucoside, and acetylated chrysophanol glucoside.
[4]. Revandchinone-1, 3, and 4 exhibited significant antifungal activity against Aspergillus niger and Rhizopus oryzae and antibacterial activity against gram positive (Bacillus subtilis, Bacillus sphaericus, and Staphylococcus aureus) and gram negative (Klebsiella aerogenes, Chromobacterium violaceum, and Pseudomonas aeruginosa) bacteria [5]. Most of the compounds have been evaluated for their medicinal activities. Hydroxyl anthraquinones and their glycosides have been found effective against animal pathogens, namely, Candida albicans, Aspergillus fumigatus,Cryptococcus neoformans, and Trichophyton mentagrophytes [6, 7]. The petroleum ether and chloroform extracts of the rhizomes have been reported to exhibit moderate antifungal and antibacterial activities; the benzene extract of R. emodi inhibited the growth of H. pylori [8].

However, only few reports are available on the agricultural pest control use of R. emodi extracts including nematode and antioxidant potential evaluation by DPPH radical scavenging activity. In the literature several methods have been reported about efficient extractions and HPLC analysis of R. emodi [9]. But, we have standardized accelerated solvent extraction (ASE) method for efficient extraction of bioactive constituents of R. emodi and compared it with Soxhlet extraction method. This was followed by separation on flash chromatography and purity check by analytical reverse phase high pressure liquid chromatography (HPLC). LC-MS/MS method in ESI mode has been employed in the direct infusion mode for the detection and confirmation of the structure of isolated, purified products which were further characterized by their spectroscopic details using infrared (IR) and $^1$H NMR spectroscopy.

2. Materials and Methods

2.1. Chemicals and Plant Materials. Laboratory and analytical grade chemicals, reagents, and solvents were locally procured. Quercetin, gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were procured from Merck India Ltd. Authentic samples of the rhizomes of Rheum emodi were procured, Delhi [10]. The nematode Meloidogyne incognita (root-knot nematode) cultures were obtained, cultured, and identified from the farms of the Division of Nematology, IARI, New Delhi.

2.2. Extraction and Isolation

2.2.1. Accelerated Solvent Extraction. The extraction was carried out using a Dionex ASE 300 (Sunnyvale, CA, USA) accelerated solvent extractor. A 50 g portion of the root powder of R. emodi was packed tightly in a 100 mL stainless steel vessel and extracted with methanol at different temperatures (40, 50, and 60°C). The extractions were performed under pressure at 10.34 Mpa, with 5 min equilibration, 5 min static time, 60% purge volume, and a 60 sec purge for a total of three cycles. About 80–100 mL of solvent was collected in each extraction. The solvent was removed under reduced pressure at 40°C in a rotary evaporator (Heidolph, Germany) to obtain dark yellow coloured viscous residue. All the operations were repeated thrice and the extracts were kept at −20°C until analysis.

2.2.2. Soxhlet Extraction. R. emodi rhizome powder (150 gm) was Soxhlet extracted with methanol (500 mL) for 8 hours. It was concentrated under reduced pressure and then partitioned with chloroform and ethyl acetate. The chloroform extract gave positive test for anthraquinones and showed four bands on the TLC plate. Four bands were scrapped and eluted with methanol. The purity of individual bands was checked by HPLC.

2.3. Chromatography and Spectroscopy

2.3.1. Thin Layer Chromatography (TLC). Activated silica gel coated plates (6 cm × 20 cm) of 0.25 mm thickness were used. Preparative TLC was performed on silica gel coated glass plates (20 cm × 20 cm) of 0.25 mm thickness. The preparative plates were developed in different solvent combinations like (i) petroleum ether : ethyl acetate : formic acid (2 : 8 : 0.1) and (ii) hexane : chloroform : acetic acid (9 : 1 : 0.1). Plates were air-dried and visualized with iodine vapor. The individual bands were scraped and extracted with methanol and the solvent evaporated under vacuo to obtain the compounds.

2.3.2. Flash Chromatography. Methanol fraction of R. emodi was also fractioned using flash chromatography (Biotage, Isolera®). The elution was done automatically by programming the instrument with hexane-chloroform (CHCl$_3$) [(3:1), (2:1), (1:1), (1:2) v/v], CHCl$_3$ and ethyl acetate (EtOAc), and CHCl$_3$-methanol (MeOH) [(9:1), (8:2), (7:3) v/v]. The fractions of same R$_f$ (TLC) were combined and concentrated in a rotary evaporator at 40°C to obtain sufficient quantity of pure anthraquinones like emodin, aloe-emodin, physcion, chrysophanol, and so forth.

2.3.3. High Performance Liquid Chromatography (HPLC). Analytical reverse phase HPLC (WATERS e2695) fitted with quaternary gradient pump, autosampler, controller Empower 2 software, waters 2998 PDA detector, and waters spherisorb® 5 μm ODS2 (4.6 × 250 mm) analytical column was used for all the analysis. Anthraquinones were separated under gradient elution at a flow rate of 1 mL min$^{-1}$ using a mobile phase of ACN : methanol (95 : 5) and water (0.2% CH$_3$COOH v/v). A 20 μL volume of sample was injected each time and peaks were detected at λ$_{max}$ of 290. The retention time (R$_t$) for each compound was recorded. Water used in HPLC analysis was obtained from Millipore water purifier system with resistivity of 18.2 MΩcm.

2.3.4. Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR was performed by Bruker Alpha making KBr palates, using Hydraulic press and the data processed using Opus 65 software.

2.3.5. Nuclear Magnetic Resonance Spectroscopy. The $^1$H spectra of the compounds were recorded on Bruker (400 MHz)
instrument. Samples were dissolved in deuterated chloroform (CDCl₃), dimethyl sulfoxide (DMSO), or carbon tetrachloride (CCl₄) as per the requirement. Tetramethylsiline (TMS) was used as an internal standard. Chemical shifts were recorded in δ (ppm) relative to TMS.

2.3.6. Electrospray Mass Spectroscopy (ESI-MS). Liquid chromatography coupled with mass spectrometry (LC-MS) is a powerful technique to analyze complex botanical extracts [11–15]. It facilitates rapid and accurate identification of chemical compounds, especially when a pure standard is not available. The technique has been extensively used in the past for the analysis of phenolic compounds [16]. The most active extract of R. emodi was analyzed for its active constituents and their structures confirmed by electrospray mass spectroscopy (ESI-MS) in the direct infusion mode.

Mass spectroscopy was carried out using Thermo Electron spectrometer (Thermo Electron Corporation, USA). Detection of mass was done by electron-spray ionization (ESI) source with Finnigan LCQ tune plus program fitted with MAX-detector. Xcalibur software was used for the purpose of identification, quantification, and fragmentation of required masses. The MS parameters optimized in direct infusion mode were spray voltage 3.5 to 5 kV, sheath gas flow rate 10 mL min⁻¹, auxiliary gas flow rate 5 mL min⁻¹, spray current 0.5, capillary temperature 225°C, capillary voltage 20–35 V, and tube lens offset 40–65. The mass spectra were recorded in negative ion mode (Figure 1).

2.4. Characterization of R. emodi Anthraquinones. The preparative thin layer chromatography of the chloroform extracts afforded four compounds: chrysophanol, physcion, emodin, and aloe-emodin. The bands on the TLC were scrapped and eluted with methanol. The purity of the individual bands was checked by TLC followed by their analysis by HPLC (Figure 2).

The spectral characteristics of pure anthraquinones are as follows.

Chrysophanol. The first compound was crystallized from chloroform-acetone as yellow powder [m.p. 194°C, Rf 0.9 in hexane + ethyl acetate + formic acid (2 + 8 + 0.1 by volume)]. \(^{1}H\text{-NMR}\) (CDCl₃) δ: 2.45 (3-H, s, H-3), 7.15 (1H, d, H-2), 7.59 (1H, d, H-4), 7.30 (1H, dd, H-7), 7.86 (1H, dd, H-5), 7.69 (1H, d, H-6), 12.03 (1H, s, OH-1), 12.14 (1H, s, OH-8); UV (λ_max MeOH): 228, 256, 279, 287, 429 nm; IR (MeOH): 3470, 3080, 2940, 1680, 1630, 1590, 1460, 1470, 1260, 1210 cm⁻¹; ESI-MS: \(m/z\) 253.3 [M – H]⁻, 225.3 [M – 28]⁻, 209.1 [M – 28–15]⁻.

Physcion. The second compound was crystallized from chloroform to give yellow needles [m.p. 204°C, Rf 0.8 in hexane + ethyl acetate + formic acid (2 + 8 + 0.1 by volume)]. \(^{1}H\text{-NMR}\) (CDCl₃) δ: 12.13 (1H, s, OH-1), 12.33 (1H, s, OH-8), 6.69 (1H,
Emodin. The third compound was crystallized from diethyl ether + ethyl acetate (80 + 20) to give pinkish powder [m.p. 258 °C, Ref 0.40 in hexane + ethyl acetate + formic acid (2 + 8 + 0.1 by volume)]. 1H-NMR (CDCl3) δ: 12.30 (1H, s, OH-1), 12.80 (1H, s, OH-8), 6.69 (1H, d, H-2), 7.09 (1H, d, H-7), 7.37 (1H, s, H-4), 7.63 (1H, d, H-5), 5.65 (1H, H-6); IR (ν MeOH): 3390, 1675, 1630, 2950 cm−1; ESI-MS: m/z 269.2 [M − H]−, 241.3 [M − 28]− and m/z 225.2 [M − 28−17].

Aloe-Emodin. The fourth compound was crystallized from chloroform: acetone (3 + 1) as yellow needles [m.p. 220 °C, Rf 0.30 in hexane + ethyl acetate + formic acid (2 + 8 + 0.1 by volume)]. 1H-NMR (CDCl3) δ: 12.08 (1H, s, OH-1), 12.10 (1H, s, OH-8), 7.32 (1H, d, H-2), 7.35 (1H, dd, H-7), 7.69 (1H, d, H-4), 7.80 (1H, d, H-6), 7.85 (1H, dd, H-5), 4.35 (2H, H-3), 5.35 (1H, OH-3); ESI-MS: m/z 269.2 [M − H]−, 241.2 [M − 28]−; IR (ν MeOH): 3300, 3050, 2890, 1650, 1610, 1590, 1250 cm−1.

2.5. Extraction of Nematodes from Plant Root. M. incognita infected plant material was collected from the glass house of Division of Nematology, I.A.R.I., New Delhi. The infected roots of brinjal (cv. Pusa Purple Long) were washed thoroughly with tap water to remove soil adhering to the roots. Meloidogyne egg mass was collected from the brinjal under stereoscopic microscope. The egg masses were kept on double layered tissue paper supported by wire mesh and kept 2-3 days in incubator at 25–30 °C and 70% RH for hatching. The number of freshly hatched second stage (J2s) juveniles was counted per 1 mL aliquot of distilled water.

Fresh nematode suspension (1 mL) was taken in a square counting dish (marked with 24 equal squares) under stereoscopic binocular microscope. The total number of nematodes in one mL was determined by counting the root-knot juveniles in each square. This process was repeated for five times and the average was taken. Suspension of the J2s was diluted further to get approximately 25 J2s per mL.

2.6. Test Procedure

2.6.1. Antioxidant Activity. The antioxidant activity of test extracts such as CE1, CE2, CE3, and standard compounds gallic acid and quercetin was assessed by radical scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [17–19]. The diluted working solution of the test extracts and the standards (1–100 mg/L) were prepared in methanol. 1 mL of DPPH (0.002%) solution in methanol was mixed separately with 1 mL of the sample solution and the standard solution. Different concentrations (50, 100, 500, and 1000 mg/L) of test extracts and the standards were pipetted to the test tubes and volume adjusted to 3 mL with methanol. 1 mL of alcoholic DPPH (0.002%) solution was added to each sample and the samples were vortexed and then incubated in dark at room temperature for 30 min. Methanol (1 mL) with DPPH solution (0.002%, 1 mL) was used as blank. The spectrophotometric measurements at 517 nm against blank samples were made with a pair of matched quartz cuvettes using Analytik Jena UV-Vis spectrophotometer (SPCORD 200). The optical density was recorded and radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

\[
\text{Inhibition} (%) = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100.
\]

Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted between inhibition percentages against extract concentration with the help of statistical package (GW BASIC).

2.6.2. Nematicidal Activity. Root-knot nematode suspension (2 mL) containing 50 juveniles was taken in petri plates; an equal volume of the test solutions was added separately to obtain the desired test concentrations of 200, 100, 50, and 25 mgL−1, respectively. Each treatment was replicated thrice separately for 24, 48, and 72 hours. For each treatment a control was taken by adding 2 mL of nematode suspension with equal volume of ethanol-Tween 80 emulsified water at 25–30°C. After 24 h of exposure, the petri plates for each treatment were observed under stereoscopic binocular microscope for determining mortality. A revival test was performed for each treatment by decanting the test solvents and adding distilled water to the petri plates. After 48 h of exposure readings were taken again for each treatment. The number of immobile juveniles counted and deducted from the number of immobile juveniles obtained in the previous reading. Similar process was followed after 72 hr exposure time. The number of immobile juveniles in each plate was calculated and the corrected percent mortality was calculated by using the following Abbott's formula (1925) [20]:

\[
\text{Corrected Mortality} (%) = \left( \frac{T - C}{100 - C} \right) \times 100,
\]

where T = total mortality in treatment and C = total mortality in control.

The mortality data were subjected to Probit analysis. LC50 value (mgL−1) was calculated from the concentration (mgL−1) and the corresponding % corrected mortality data with the help of statistical package (GW BASIC).

3. Results and Discussion

3.1. Extraction and Characterization of Anthraquinones. The mass spectra of four hydroxyanthraquinones identified in
rhubarb are shown in Figure 3. ESI-MS of anthraquinones provides more structural information than the APCI-MS [21, 22]. In the ESI-MS spectrum of chrysophanol, a small molecular ion peak at \( m/z \) 253 [M – H] and a stable large fragment ion at \( m/z \) 252 [M – 2H] ion were observed as a result of deprotonation of one or two protons from phenolic-

OH groups. A fragment ion at \( m/z \) 225 originated as a result of the direct loss of CO from [M – H]. The \( m/z \) 225 ion was stable and did not result in further fragmentation. The CO elimination may preferably originate from C-10, since the carbonyl group at C-9 has intramolecular hydrogen bonding with the \( \alpha \)-hydroxyl groups at C-1 and C-8 and is therefore difficult to be cleaved. Emodin and aloe-emodin are isomers and can be differentiated by their ESI-MS spectra. Both the compounds exhibit deprotonated molecular ion peak at \( m/z \) 269 [M – H]. The fragmentation of emodin was initiated by the elimination of CO to produce \( m/z \) 241, followed by loss of one hydroxyl group to give fragment ion at \( m/z \) 225. The stable \( m/z \) 269.3 [M – H] ion of aloe-emodin does not lead to formation of any peak at \( m/z \) 225 rather, its fragmentation formed a peak at \( m/z \) 253.3 [M–OH]. Physcion showed a prominent molecular ion peak at \( m/z \) 283 [M – H]. It contained a methyl group that easily eliminates 15 mass units to give a significant ion at \( m/z \) 269.2 followed by elimination of methoxy group \( m/z \) 253.3 [M – OCH3]. This ion could further lose one molecule of \( \text{H}_2\text{O} \) to produce fragment ion at \( m/z \) 235.2.

**Chrysophanol.** The first compound was crystallized from chloroform-acetone as yellow leaflets, m. p. 194°C. Rf is 0.9 (hexane: ethyl acetate: formic acid; 2:8:0.1). The IR (KBr) \( \nu_{\text{max}} \) of the purified metabolite showed a strong hydroxyl group absorption band at 3470 cm\(^{-1}\), together with two carbonyl group absorption bands at 1680 and 1633 cm\(^{-1}\). It further shows absorption maxima at UV (\( \lambda_{\text{MeOH}} \max \)) 228, 256, 279, 287, and 429 nm. Additionally, its \(^1\)H NMR spectrum showed two sharp singlets at \( \delta \) (ppm) 12.03 (OH-1) and 12.14 (OH-8) characteristic of two chelated hydroxyl groups and signals for five aromatic protons, 7.15 (1H, d, H-2), 7.59 (1H, d, H-4), 7.30 (1H, dd, H-7), 7.86 (1H, dd, H-5), and 7.69 (1H, d, H-6) and for the aromatic methyl group of an anthraquinone 2.45 (s, H-3). On the basis of its spectroscopic data, and by comparing it with those reported in the literature, the molecule was identified as chrysophanol (1,8-dihydroxy-3-methylanthracene-9,10-dione).

**Physcion.** The second compound was crystallized from chloroform to give yellow needles, m.p. 204°C. Rf is 0.8 in hexane: ethyl acetate: formic acid (2:8:0.1) and 0.85 in benzene: chloroform: acetic acid (9:1:0.1). The IR (KBr)

**Figure 3:** ESI-MS of rhubarb anthraquinones.
γ_max of the purified metabolite showed a strong carbonyl group absorption bands at 1675 and 1630 cm\(^{-1}\). It further shows absorption maxima at UV (\(\lambda_{\text{MeOH max}}\)): 255, 250, and 430 nm. Additionally, its \(^1\)H NMR spectrum showed two sharp singlets at \(\delta\)12.13 and 12.33 and signals for five aromatic protons: \(\delta\) 2.46 (CH\(_3\)), 6.69(1H), 7.09 (1H), 7.37 (1H), 7.63 (1H) and for the aromatic methoxy group of an anthraquinone \(\delta\) 3.94 (OCH\(_3\)). On the basis of its spectroscopic data, and by comparing it with those reported in the literature, the molecule was identified as physcion (1,8-dihydroxy-6-methyl-3-methoxyanthracene-9,10-dione). The fourth compound was crystallized from hexane: ethyl acetate (80 : 20) to give pinkish leaflets, m.p. 258°C. The RF is 0.40 (hexane : ethyl acetate : formic acid; 2 : 8 : 0.1) and 0.45. (hexane : chloroform : acetic acid (9 : 1 : 0.1). Emodin showed absorption maxima at 437, 299, and 260 nm. IR of emodin showed frequency at 3390 cm\(^{-1}\) assigned to hydroxyl groups. Characteristic bands for anthraquinones at 1675 cm\(^{-1}\) and 1631 cm\(^{-1}\) were obtained which is assigned to the free carbonyl group and the conjugated carbonyl group stretching frequencies, respectively. The observed frequency at 1475 cm\(^{-1}\) is assigned to a skeletal ring stretching frequency. Its \(^1\)H NMR spectrum showed two sharp singlet at \(\delta\)12.30 and \(\delta\) 12.80 and signals for four aromatic protons \(\delta\) 6.69 (1H), 7.09 (1H), 7.37 (1H), 7.63 (1H)) and for the aromatic methyl group of an anthraquinone \(\delta\) 2.46 (CH\(_3\)). On the basis of its spectroscopic data, molecule was identified as emodin (1, 8-dihydroxy-6-methyl-3-hydroxyanthracene-9,10-dione).

**Aloe-Emodin.** The third compound was crystallized from chloroform: acetone (3 : 1) as yellow needles, m.p. 220°C. The RF is 0.30 (hexane : ethyl acetate : formic acid; 2 : 8 : 0.1) and 0.32 (hexane : chloroform : acetic acid (9 : 1 : 0.1). Aloe- emodin showed absorption maxima at 225, 224, 276, 287, and 457 nm, respectively. IR of aloe-emodin shows frequency at 3390 cm\(^{-1}\) assigned to hydroxyl groups. Characteristic bands for anthraquinones are 1675 cm\(^{-1}\) and 1631 cm\(^{-1}\) assigned to the free carbonyl group and the conjugated carbonyl group stretching frequencies, respectively. The observed frequency at 1475 cm\(^{-1}\) is assigned to a skeletal ring stretching frequency. Its \(^1\)H NMR spectrum showed two sharp singlets at \(\delta\)12.08 and 12.10 and signals for five aromatic protons: \(\delta\) 7.32 (1H, 5-H), 7.35 (1H, 4-H), 7.69 (1H, 6-H), 7.80 (1H, 7-H), 7.85 (1H, 2-H)] and for the characterstics aromatic methylene group at \(\delta\) 4.85 (CH\(_3\)) and for aromatic hydroxyl at \(\delta\) 5.35(OH) of an anthraquinone. On the basis of its spectroscopic data, the molecule was identified as aloe-emodin (1, 8-dihydroxy-3-hydroxymethylanthracene-9,10-dione).

**Table 1:** IC\(_{50}\) (mg/L) and DPPH scavenging activity (%) of *Rheum emodi* (rhubarb) extracts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% DPPH scavenging activity</th>
<th>(\chi^2) obs, (3d.f. 95%)</th>
<th>Regression equation</th>
<th>Fiducial limit</th>
<th>IC(_{50}) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE1</td>
<td>20.12</td>
<td>5 mg/L</td>
<td>87.95</td>
<td>1.14</td>
<td>Y = 3.18 + 1.44X</td>
</tr>
<tr>
<td>CE2</td>
<td>21.56</td>
<td>10 mg/L</td>
<td>88.33</td>
<td>5.94</td>
<td>Y = 3.09 + 1.48X</td>
</tr>
<tr>
<td>CE3</td>
<td>19.80</td>
<td>50 mg/L</td>
<td>85.59</td>
<td>0.971</td>
<td>Y = 3.15 + 1.41X</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>43.38</td>
<td>100 mg/L</td>
<td>91.41</td>
<td>5.59</td>
<td>Y = 4.32 + 0.98X</td>
</tr>
<tr>
<td>Quercetin hydrate</td>
<td>31.79</td>
<td>5 mg/L</td>
<td>92.43</td>
<td>2.96</td>
<td>Y = 3.59 + 1.34X</td>
</tr>
</tbody>
</table>

Results shown are means of three experiments.

3.2. Antioxidant Activity. The extracts of *R. emodi* were analyzed for antioxidant activity by using DPPH free radical scavenging activity. The anthraquinone derivatives, such as aloe-emodin, emodin, rhein, chrysophanol, and physcion, are reported to possess antiangiogenic activity, by preventing blood vessel formation in zebra-fish embryos [24]. The anticancer effect of aloe-emodin has been established in two human cancer cell lines, Hep G2 and Hep 3B. Aloe-emodin inhibited cell proliferation and induced apoptosis in both examined cell lines by different antiproliferative mechanisms [25]. In our study all the extracts showed a concentration dependent scavenging of DPPH radicals (Figure 3). CE1 with (IC\(_{50}\) 18.28 mg/mL) was most active, followed by ethyl CE2 (IC\(_{50}\) 19.37 mg/mL) and CE3 (IC\(_{50}\) 20.27 mg/mL) (Table 1). All the extracts were comparatively less active than both quercetin hydrate (QH) (4.95 mg/L) and gallic acid (GA) (11.26 mg/L) in quenching DPPH radical than standard antioxidants. The extracts contained a high number of phenolic compounds, which were found to have significant positive correlation with free radicals (DPPH and OH) scavenging efficacies. The results were in agreement with those of [26] who found the methanolic extract to be a more active radical scavenger than aqueous extract.

The HPLC analysis had shown the methanolic extract of *R. emodi* rhizomes to be rich in the major anthraquinones.
and their glucosides, namely, emodin, aloe-emodin, chrysophanol, physcion, emodin glucoside, chrysophanol glucoside, and revandchinone, whereas ethyl acetate and chloroform extract was found to be low in aloe-emodin and other glucosides. The lower IC$_{50}$ value for methanolic extract in free radical scavenging activity of DPPH is attributed to the fact that the extract is rich in bioactive constituents including anthraquinones. However, our results are in contrast to the study [4] where the compounds like marsupsin and maesopsin obtained from the rhizome/root extracts of *R. emodi* are found to possess antioxidant activity, whereas chrysophanol, physcion, and emodin as well as their 8-O-glucosides were found to be inactive.

3.3. Nematicidal Activity. After 72 hr of exposure and observation time CE1 (ED$_{50}$ 54.84 mg/L) was most active followed by CE2 (ED$_{50}$ 98.10 mg/L) and CE3 (135.23 mg/L). Among the pure anthraquinones chrysophanol (ED$_{50}$ 102.59 mg/L)
### Table 2: Antinemic activity (ED$_{50}$) of isolated anthraquinones and solvent extractives of *Rheum emodi* against *j*$_2$s of *Meloidogyne incognita* after 72 hrs.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\chi^2_{obs}$ (3d.f, 95%)</th>
<th>Regression equation</th>
<th>ED$_{50}$ (mg/L)</th>
<th>Fiducial limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE1</td>
<td>1.91</td>
<td>$Y = 2.74 + 1.30X$</td>
<td>54.84</td>
<td>44.51–67.57</td>
</tr>
<tr>
<td>CE2</td>
<td>6.73</td>
<td>$Y = 2.73 + 1.13X$</td>
<td>98.10</td>
<td>74.34–129.46</td>
</tr>
<tr>
<td>CE3</td>
<td>6.47</td>
<td>$Y = 2.26 + 1.27X$</td>
<td>135.23</td>
<td>101.27–180.58</td>
</tr>
<tr>
<td>Physcion</td>
<td>2.50</td>
<td>$Y = 2.33 + 1.32X$</td>
<td>102.61</td>
<td>82.80–136.25</td>
</tr>
<tr>
<td>Aloe-emodin</td>
<td>7.39</td>
<td>$Y = 2.25 + 1.27X$</td>
<td>148.50</td>
<td>109.17–202.01</td>
</tr>
<tr>
<td>Chrysophanol</td>
<td>2.15</td>
<td>$Y = 2.03 + 1.42X$</td>
<td>102.59</td>
<td>81.50–129.15</td>
</tr>
<tr>
<td>Emodin</td>
<td>2.62</td>
<td>$Y = 2.03 + 1.38X$</td>
<td>139.95</td>
<td>106.64–183.6</td>
</tr>
<tr>
<td>Aza (20%)</td>
<td>0.42</td>
<td>$Y = 3.42 + 0.76X$</td>
<td>112.70</td>
<td>73.14–173.67</td>
</tr>
</tbody>
</table>

The present study conclusively demonstrated that the anthraquinones and other phenolics of *R. emodi* especially in the methanolic extract (CE1) have great potential and can be suitably utilized into environmentally benign pest control strategies. The potential antioxidant activity of the *R. emodi* constituents as revealed by the DPPH assay strengthens the importance of anthraquinones in ethno-medical use as well as in contemporary medicine. Further LC-MS analysis revealed that the activity of CE1 was mainly due to the synergistic combinations of its anthraquinone and their glucosides along with other phenolics. The constituents can suitably become one of the lead molecules to be further exploited in various spheres of pest and disease control mechanisms after suitable clinical testing.

### 4. Conclusion

The authors declare that there is no conflict of interests regarding the publication of this paper.

**Conflict of Interests**

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