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Ruchi Singh
Department of Biological Sciences, CBSH, G. B. Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar. Uttarakhand, India

Preeti Chaturvedi
Department of Biological Sciences, CBSH, G. B. Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar. Uttarakhand, India

Correspondence
Ruchi Singh
Department of Biological Sciences, CBSH, G. B. Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar. Uttarakhand, India

Phytochemical screening and determination of antioxidant activity in callus and different parts of *Rheum emodi* Wall ex. messin

Ruchi Singh and Preeti Chaturvedi

Abstract
Natural products are the main source of antioxidants that protect human body from oxidative damage. There are number of plants which are rich repository of the antioxidants. These plants need to be assessed scientifically for use as natural source of antioxidants. Hence, the objective of the present study was aimed to evaluate the phytochemical profile of a highly useful medicinal herb *i.e*., *Rheum emodi*. The Present research explores the phytochemical profile including phenolic content, flavonoid content and antioxidant activity in methanolic extracts of rhizomes, fruits, leaves and callus of *R. emodi*. Data was analysed statistically by ANOVA (using STPR 2) and level of significance was determined by DMRT (using SPPS version 16). Phytochemical screening showed the presence of plethora of phytochemicals like phenols, flavonoids, alkaloids, carbohydrates, proteins, anthraquinones, quinones and glycosides. All extracts of *R. emodi* exhibited DPPH radical scavenging activity (%) and total antioxidant capacity in dose dependent manner with high level of significance. DPPH free radical scavenging activity was highest in rhizome (94.57 ± 0.26) and lowest in callus (70.86± 0.85) at 100 µg conc. Likewise, the fruits and callus showed maximum (24.76 ± 0.23) and minimum (5.52 ± 0.063) total antioxidant capacity (µg AAE/mg extract) respectively. Total phenolic content (µgGAE/mg extract) and total flavonoid content (µgQE/mg extract) was maximum in fruits (124.64 ± 0.81) and leaves (165 ± 0.57) respectively whereas callus exhibited minimum TPC (17.23 ± 0.50) and TFC (18.06 ± 0.21) values. All extracts of *R. emodi* showed positive correlation between total antioxidant capacity and phenolic content. All the parts of *R. emodi* used in the present study possessed significant antioxidant activity. Based on the study, it is recommended that not only the rhizomes, but aerial parts as well as calli can also be utilised as valuable sources of medicinally important antioxidants in pharmaceutical industries.

Keywords: *Rheum emodi*, phytochemical screening, TPC (Total phenolic content), TFC (Total flavonoid content), antioxidant activity

Introduction
Rheum emodi Wall ex. Meissn belonging to family Polygonaceae is one of the important medicinal herbs widely used in Ayurvedic and Unani system of medicine from ancient times[1]. This species of *Rheum* is endemic to western and central Himalayan region. Rhizome and roots of the plant are the important parts that are used throughout the world for curing various ailments such as jaundice, headache, migraine, paralysis, sciatica, asthma, diarrhea and liver disorders etc [2, 3]. Important phytoconstituents of the plant include anthraquinones (emodin, aloe-emodin, physcion, rhein, and chrysophanol) and stilbenes (piceatannol, resveratrol) which possess anti-cancerous activities against breast cancer, prostate cancer, colon cancer, leukemia and lymphoma [4, 5]. Other phytoconstituents, oxanthrone esters (revandchinone 1, revandchinone 2, revandchinone 3 and revandchinone 4) show significant antimicrobial activities against various microorganisms *viz*. *Bacillus subtilis*, *Staphylococcus aureus* (gram +ve), *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Chromobacterium violaceum* (gram –ve), *Aspergillus niger* and *Rhizopus oryzae* [6]. Ethanolic extracts of the rhizome exhibit gastroprotective and antidiabetic activities [7].

Most of the plants show their medicinal properties due to the presence of phenolic compounds that also exhibit antioxidant activity. Antioxidants are substances that prevent, delay or remove the oxidative damage caused to target molecules even at relatively low concentration by reducing the level of the Reactive Oxygen Species (ROS) or free radicals [8]. ROS are generally produced as a product of cellular metabolism through electron transport chain in mitochondria, microsomal oxidation in endoplasmic reticulum, myeloperoxidase in phagocytes and also through environmental stresses such as UV radiations, drought, chilling and salinity [9, 10]. Large numbers of synthetic antioxidants are available commercially, but most of these antioxidants display some side effects, due to which the researchers are now focussing on finding the natural sources of antioxidants [11, 12].

The present study, assessed the phytochemical screening, total phenols, total flavonoids and antioxidant activity in different parts (Rhizome, fruit, leaf and callus) of *R. emodi* which earlier focussed only in rhizomes. A perusal of the earlier studies done on this plant, reveals that this is the first report on phytochemical screening, total phenols, total flavonoids and antioxidant activity employing aerial parts and callus of *R. emodi*.

Materials and Methods

Sample collection

The plant material (rhizome and fruits) of *R. emodi* was collected from Bagheswar district (29º51'0"N 79º46'0"E, 1,004m) of Uttarakhand in the month of March, 2016. Rhizomes were grown under controlled environment facility available in College of Basic Sciences & Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, for further studies.

Leaf explants were obtained from the pot grown plants (2-3 month old), were surface sterilised and were inoculated on MS medium supplemented with NAA (5.0 µM) + BAP (10.0 µM) for callus induction (Fig. 1A & B). All the chemicals used in the present study were of analytical grade and were procured from Merck and Hi Media Laboratories Pvt. Ltd, Mumbai, India.

Preparation of plant extract

The plant materials (Rhizomes, fruits, leaves, and callus) were washed 2-3 times with running tap water and shade dried. Thereafter, they were crushed separately to fine powder and 2 g each of the fine powder was subjected to extraction in 250 ml of methanol by using Soxhlet apparatus (Khera Instruments Pvt. Ltd., Delhi, India). The liquid extract was evaporated in a rotary evaporator (U-Tech, Star Scientific Instruments, Delhi, India) to obtain solid mass which was stored for further analysis.

Phytochemical analysis

The extracts were redissolved in methanol to make stock soln. (1mg/ml). Preliminary phytochemical analysis of methanolic extracts of *R. emodi* was carried out according to the standard methods [13, 14].

Antioxidant activity by DPPH scavenging assay

Antioxidant activity of plant extract was determined by using DPPH radical scavenging protocol given by William *et al.* [15] with some minor modifications. Different conc. (20 - 100 µg/ml) of plant extracts and BHT (reference sample) were prepared in methanol. The reaction mixture comprised of 0.004 % of DPPH soln. (1ml) and 1 ml of different conc. of plant extract and BHT soln. respectively. It was incubated in dark for 30 min. and the absorbance was recorded at 517 nm against blank. The reaction mixture without extract was taken as control and the capability of scavenging the DPPH radical was calculated by using the following equation:

DPPH radical scavenging activity (%) = $(A_c - A_t/A_c) \times 100$

Where A_c was absorbance of control reaction and A_t was absorbance of the test sample or BHT.

Total antioxidant Capacity (TAC)

Total antioxidant capacity was evaluated by using phosphomolybdenum method suggested by Prieto *et al.* [16]

Total Phenolic Content (TPC)

The total phenolic content was determined by using Folin–Ciocalteau method (Johnson and Schaal) [17] with some minor modifications. Approximately, 0.2 ml of Folin – Ciocalteau reagent was added to 500 µg/ml of sample extract. After 5 min. 0.5 ml of 7 % saturated Na_2CO_3 was added and volume of reaction mixture was raised to 5ml by adding dist. water. This reaction mixture was incubated for 1 hr at room temperature and absorbance was taken at 765 nm against blank. A standard curve was prepared using different conc. (20 µg/ml – 120 µg/ml) of Gallic acid. TPC values were expressed as µg gallic acid equivalent (GAE) /mg extract from the standard curve.

Total Flavonoid Content (TFC)

The total flavonoid content was determined on the basis of the method suggested by Djeridane *et al.* [18] with some minor modifications. About 0.5 ml of 2 % aluminium chloride ($AlCl_3$) was mixed with 500 µg/ml of the sample extract and vortexed. The reaction mixture was incubated for 1 hr and absorbance was recorded at 420 nm against blank. Quercetin was used for constructing the standard curve and TFC values were expressed as µg quercetin equivalent (QE)/mg extract.

Statistical Analysis

All the experiments were performed in triplicates and statistical analysis was performed by STPR 2. Significant differences between the means were determined by DMRT using SPSS 16.0 version at 5% level of significance and the correlation among the data was obtained by using correlation coefficient (r^2).

Results and Discussion

Phytochemical Screening

All the plant parts of *R. emodi* (rhizomes, fruits, leaves) as well as *in vitro* grown callus showed the presence of various phytochemicals *viz*. flavonoids, phenols, tannins, alkaloids, glycosides, carbohydrates, proteins, anthraquinones and quinines. (Table 1)

DPPH Radical Scavenging Activity

All the extracts of *R. emodi* except fruit extract showed DPPH radical scavenging activity (%) in dose dependent manner with high level of significance ($p < 0.05$). Among all the extracts, rhizomes showed highest DPPH radical scavenging activity (94.57 ± 0.26) followed by fruits (92.80± 0.07), leaves (77.07±0.35) and callus (70.86± 0.85) at 100 µg conc. (Fig 2). Similar results were reported by Rajkumar *et al.* [19] showing 90% and 20% DPPH radical scavenging activity in methanolic and aqueous extract of the rhizome of *R. emodi* at 100 µg conc. respectively. However, Kumar *et al.* [20] reported 50 and 90% DPPH radical scavenging activity in 1000 µg of hot and cold chloroform extracts of rhizome of *R. emodi* respectively. DPPH radical scavenging activity in methanolic extracts of different plants were reported as *Hemidesmus indicus* (stem) 77.0 %, *Plumbago zeylanica* (roots) 73.41 %, *Holarrhena antidysenterica* (bark) 20.88 %, *Acorus calamus* (rhizome) 6.32% [21], *Alpinia nigra*(leaves) 60% [22], *Eclipta prostrate* 80.13% [23], *Centella asiatica* (leaves) 85.73% [24]. In the current study, *R. emodi* (rhizome) showed 94.57 % DPPH radical scavenging activity at 100 µg conc. which is significantly better than the above mentioned

plants. The present study suggested that not only the rhizomes, but other parts of *R. emodi* also showed high antioxidant activities.

Total Antioxidant Capacity

All the extracts of *R. emodi* showed total antioxidant capacity in dose dependent manner with high level of significance ($p<0.05$). The fruits showed maximum total antioxidant capacity (24.76 ± 0.23µg AAE/mg extract) whereas callus showed minimum (5.52 ± 0.063 µg AAE/mg extract) at 100 µg conc. (Fig 3). Ibrahim *et al*. [25] reported 288.88 ± 9.66 µg/ml (ethanol) and 145.40 ± 5.27µg/ml (water) total antioxidant capacity in *R. rhaponticum* roots where as Phatak and Hendre [26] reported 0.363 ± 0.02 µg AAE/mg total antioxidant capacity at 100 µg conc. in *Kalanchoe pinnata*. The total antioxidant capacity of the fruits was high as compared to rhizomes that clearly projects its potential as a remarkable source of antioxidants.

Total Phenolic Content

Fruits and rhizome extracts of *R. emodi* showed **t**otal phenolic content of 124.64 ± 0.81 and 92.82 ± 0.23µgGAE/mg respectively (Fig. 4). The present study reports considerably higher TPC in fruits than in rhizomes. However, previous studies *viz*., Letowska *et al*. [27] reported 20 mg GAE/g phenolic content in methanolic extract of *R. palmatum* (root). Gupta *et al*. [28] recorded phenolic content of 6.85 and 14.51 g GAE/100g dry wt. in methanolic and aq. extracts of *R. australe* respectively. Whereas Ibrahim *et al*. [25] reported 1.115g GAE/g and 0.655g GAE/g dry wt. of total phenolic content in the ethanolic and aq. extracts of *R. raphonticum* respectively. In the present study, highly positive correlation was observed between antioxidant activity and phenolic content in all the parts of *R. emodi* as well as in the callus (Fig. 6-9).

Total Flavonoid Content

Flavonoids are one of the important group of phenolic compounds that not only possess the antioxidant activities but are also used to provide the beneficial effects to human health by possessing antiviral, anti-allergic, anti-inflammatory and anti-cancer activities. *R. emodi* showed remarkably good flavonoid content inleaves (165 ± 0.57µgQE/mg extract) and fruits (137.96 ±1.08) followed by rhizomes and callus (Fig. 5). However, flavonoid content in methanolic extract of roots were reported to be low in several medicinal plants such as *Hedychium rubrum* (21.25 ± 0.295), *H. coranarium* (2.47 ± 0.079), *H. spicatum* (4.22 ± 0.425 µg/100g) [29], *Plumbago indica* (0.65) and *Pseudarthria viscida* (0.25 mgQE/100g) [30], *Silene swertifolia* (5.64 ± 0.49), *S.gynodioca* (5.03 ± 0.144), *S. spergulifolia* (4.77 ± 0.089 mg/g) dry weight [31]. Ethanolic and aq. root extracts of *R. rhaponticum* exhibited 687± 4.58 and 149.01± 8.47 mg/100g dry wt. of total flavonoid content [25]. The present study showed 69.8 µgQE/mg flavonoid content in rhizome slower than what is present in leaves and fruits. Thus, it can be inferred that the leaves and fruits are better and efficient source of total flavonoids than the underground rhizomes. Leaves were also found as an efficient source of medicinally valuable compounds in *Picrorhiza kurroa* [32, 33].

Table 1: Pytoconstituents in methanolic extracts of *R. emodi*

S. No.	Phytoconstituents	Rhizome	Fruit	leaf	Callus
1.	**Flavonoids** H_2SO_4 Test	+	+	+	+
2.	**Phenols** Ferric Chloride Test	+	+	+	+
3.	**Alkaloids** Mayer's test Wagner's test	+ -	- -	+ -	- -
4.	**Glycosides** Salkowski test	+	+	+	+
5.	**Carbohydrates** Benedict's test Molisch's test	+ +	+ +	+ +	- +
6.	**Proteins** Ninhydrin test Xanthoproteic test	- +	- +	- +	- -
7.	**Anthraquinones**	+	+	+	-
8.	**Saponins**	-	-	-	-
9.	**Quinones** H_2SO_4 test HCl test	+ +	+ +	- -	+ -

Where, + indicates the presence and – indicates the absence of the phytoconstituents.

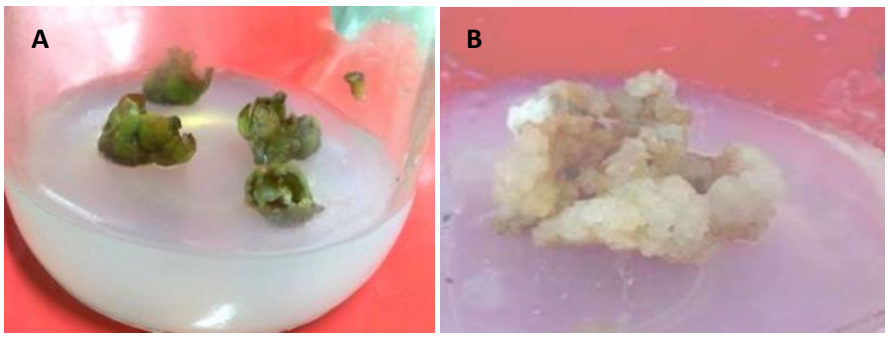

Fig 1A: Callus initiation from leaf explants of *R. emodi*, B. Callus proliferation on MS medium supplemented with NAA+BAP (µM)

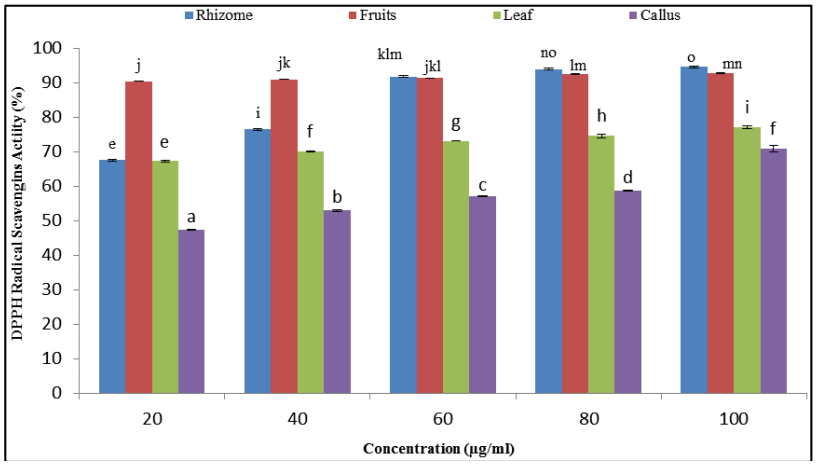

Fig 2: DPPH radical scavenging activity of methanolic extract of *R. emodi* represented as Mean ± SE. Values with different letters indicated significant difference at P<0.05 using DMRT

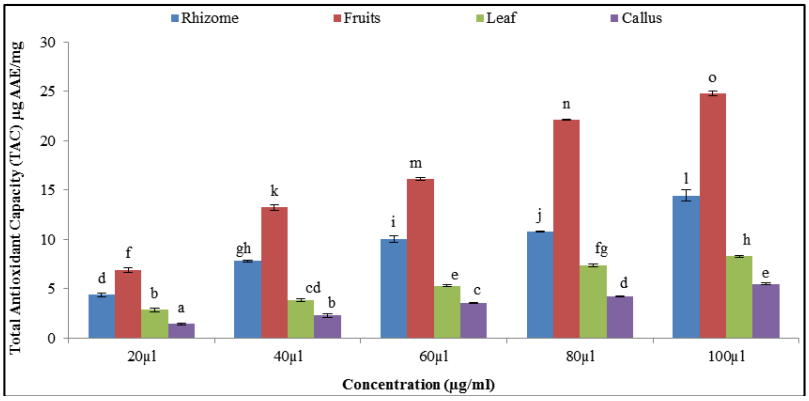

Fig 3: Total Antioxidant Capacity of methanolic extract of *R. emodi* represented as Mean ± SE. Values with different letters indicated significant difference at P<0.05 using DMRT

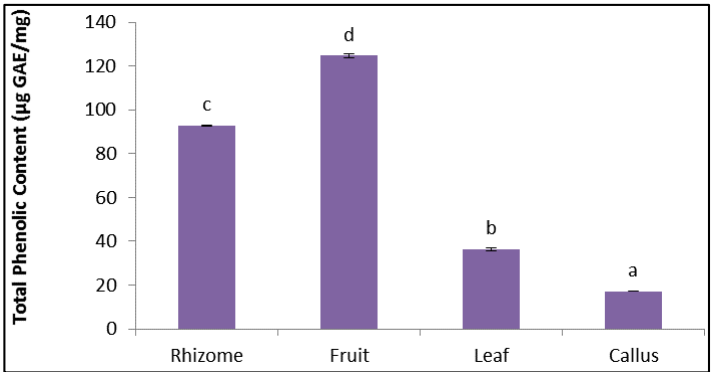

Fig 4: Total phenolic content in methanolic extracts of *R. emodi* represented as Mean ± SE. Values with different letters indicated significant difference at P<0.05 using DMRT

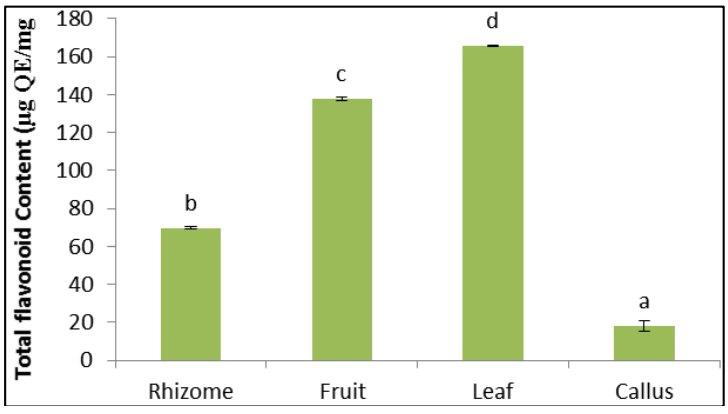

Fig 5: Total flavonoid content of methanolic extracts of *R. emodi* represented by Mean ± SE. Values with different letters indicated significant difference at P<0.05 using DMRT

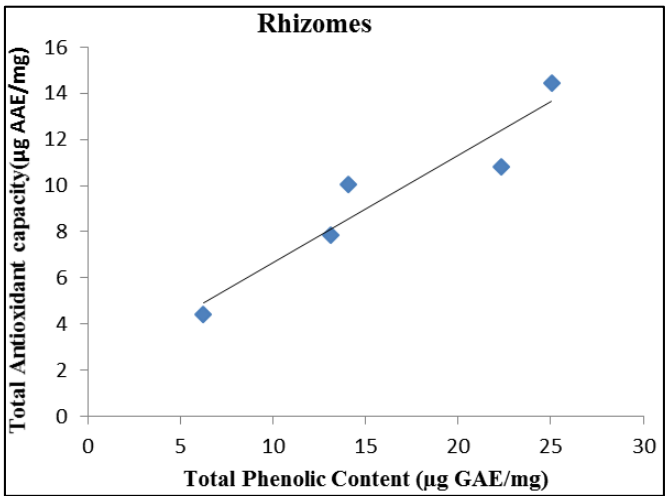

Fig 6: Correlation between the total antioxidant capacity and total phenol content of rhizomes in *R. emodi*. (Coefficient of correlation (r^2) = 0.896)

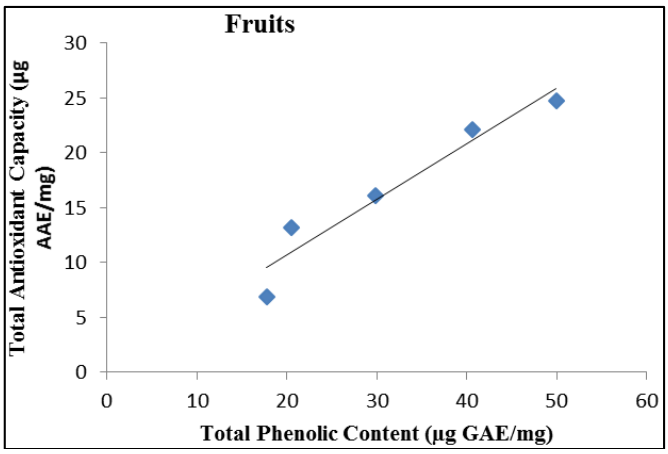

Fig 7: Correlation between the total antioxidant capacity and total phenol content in fruits of *R. emodi* (coefficient of correlation (r^2) = 0.928)

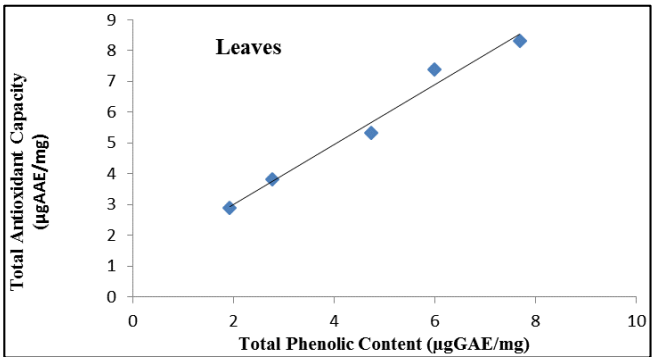

Fig 8: Correlation between the total antioxidant capacity and total phenol content in leaves of *R. emodi* (coefficient of correlation (r^2) = 0.979)

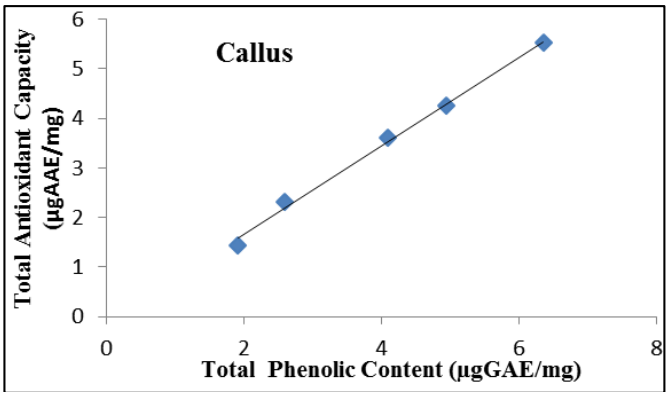

Fig 9: Correlation between the total antioxidant capacity and total phenol content in callus of *R. emodi* (coefficient of correlation (r^2) = 0.996)

Conclusion

The present study reveals that *R. emodi* possesses various secondary metabolites *viz.* alkaloids, glycosides, proteins, carbohydrates, anthraquinone and quinines along with possesses high phenolic and flavonoid content due to which it exhibits remarkably good antioxidant potential. Moreover, the analysis clearly suggests the presence of good amount of these medicinally valuable metabolites in aerial parts like leaves and fruits that can provide an efficient and better means of sustainable harvest without uprooting the plants and affecting its diversity.

Significance Statement

This study detected the phytochemical and antioxidant potential of methanolic extract of rhizomes, fruits, leaves and callus of *R. emodi* that can be beneficial for protecting the human body against various diseases. The study is a significant step towards attracting the attention of pharmaceutical industry to shift their focus from traditionally used underground rhizomes to aerial parts of *R. emodi* for sustainable and judicious yield of phytochemicals.

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