

Comparative Phytochemical Profiling and Antioxidant Activity of Selected Plants of the Genus *Rotheca* Raf. (Lamiaceae)

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<http://dx.doi.org/10.13005/bbra/3568>

(Received: 25 May 2026; accepted: 30 August 2026)

Comparative phytochemical screening and antioxidant evaluation were performed on leaf extracts of *Rotheca serrata* (L.) Steane & Mabb. and *Rotheca microphylla* (Blume) Callm. & Phillipson (Lamiaceae). Standard qualitative assays indicated the presence of alkaloids, tannins, flavonoids, glycosides, sugars, anthraquinones, steroids, terpenoids and amino acids in both species. Antioxidant activity was assessed using DPPH radical scavenging, nitric oxide scavenging, phosphomolybdenum reducing power and ferric-reducing antioxidant power (FRAP) assays. In the DPPH assay, IC₅₀ values were 66.1 µg·mL⁻¹ for *R. serrata* and 74.6 µg·mL⁻¹ for *R. microphylla*; in the nitric oxide assay, IC₅₀ values were 41.7 µg·mL⁻¹ and 57.6 µg·mL⁻¹, respectively, demonstrating greater radical-scavenging potency for *R. serrata*. In reducing-power assays, *R. microphylla* exhibited higher phosphomolybdenum reducing capacity, whereas *R. serrata* showed stronger FRAP activity. Total phenolic content measured by the Folin–Ciocalteu method was higher in *R. serrata* (746.26 ± 8.42 µg GAE·g⁻¹ extract) than in *R. microphylla* (661.58 ± 13.08 µg GAE·g⁻¹ extract), which correlates with the marginally greater overall antioxidant activity observed for *R. serrata*. These findings identify both *Rotheca* species as promising sources of natural antioxidants. The study's novelty lies in providing the first comparative antioxidant and phytochemical profiling of *R. serrata* and *R. microphylla*, establishing a quantitative link between phenolic content and antioxidant efficacy and generating baseline data that support isolation and structural elucidation of bioactive constituents and subsequent *in vivo* and mechanistic investigations. The results have potential implications for the development of plant-derived antioxidant agents and for ethnopharmacological validation of *Rotheca* species.

Keywords: Antioxidants, *Clerodendrum serratum*, Phytochemistry, *Rotheca microphylla*, *Rotheca serrata*, Total phenolic content.

Members of the plant kingdom produce a wide array of secondary metabolites: alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids and others that are responsible for their medicinal, culinary and traditional uses. These compounds contribute not only to organoleptic properties but also to plant defence, pollination, and allelopathy,

and they serve as a rich source of lead molecules for drug discovery. The Lamiaceae is a largely aromatic family with many species of medicinal and ethnobotanical importance. Within this family, the genus *Rotheca* Raf. (APG classification) comprises roughly 36 species distributed across tropical and subtropical Asia and Africa. In Kerala, India,

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two *Rothea* species occur: *Rothea serrata* (L.) Steane & Mabb., the type species widely cited for therapeutic applications,¹ and *Rothea microphylla* (Blume) Callm. & Phillipson, commonly cultivated as an ornamental, though reported as medicinal in Myanmar and parts of Africa.² Ethnobotanical records and experimental studies document diverse traditional uses of *R. serrata* for treatment of fever, respiratory disorders, inflammation, infections and malaria, among them, supporting its potential as a source of bioactive phytochemicals.³⁻⁵

Reactive oxygen species and other free radicals are normal by-products of endogenous metabolism and are also generated by exogenous stressors such as radiation, pollution, and tobacco smoke. While they play physiological roles in signalling, immunity, and programmed cell death, excessive free radicals induce oxidative stress that damages DNA, proteins, and lipids and is implicated in cancer, cardiovascular disease, neurodegeneration and inflammatory disorders.⁶ Antioxidants, many of which are plant-derived phytochemicals, which neutralize free radicals and thereby mitigate oxidative damage. Phenolic compounds in particular are ubiquitous antioxidants that make an important contribution to dietary antioxidant intake and to the therapeutic properties of many plants.

Given the reported ethnopharmacological uses of *Rothea* species and the known variability of phytochemical profiles, a comparative phytochemical and antioxidant analysis of *R. serrata* and *R. microphylla* can identify bioactive constituents and inform their therapeutic and commercial potential. This study therefore examines and compares the qualitative and quantitative phytochemical composition and *in vitro* antioxidant activities of *R. serrata* and *R. microphylla* collected from Kerala, India.

MATERIALS AND METHODS

Collection of plant materials

Fresh plant materials of *Rothea serrata* (L.) Steane & Mabb. and *Rothea microphylla* (Blume) Callm. & Phillipson were collected from Chithirapuram, Idukki District, Kerala, India (10.037117° N, 77.047123° E) and Ananganadi, Palakkad District, Kerala, India (10.825292° N, 76.332659° E), respectively, during January 2024,

taxonomically identified and authenticated by Dr Rogimon P Thomas, Department of Botany, CMS College, Kottayam. Shade-dried leaves were ground into fine powder and stored in airtight containers with proper labelling for downstream uses.

Preparation of plant extracts

The methanolic and petroleum ether leaf extracts of both plants were prepared using the ultrasonication method. 10 g of leaf powder was added to 100 mL of solvent and sonicated for 20 minutes. After ultrasonication, the extracts were filtered and used for the study. The chemicals and reagents used in the study are of analytical grade and were procured from Merck and SRL.

Qualitative Phytochemical Analysis

Both methanolic and petroleum ether extracts of the plants were tested for the presence of various phytochemicals using the following standard tests.

Test for Alkaloids: Dragendorff's test

Add 2 mL of Conc. HCl to 5 mL of extract. Add 1 mL of Dragendorff's reagent to it. Appearance of an orange or red precipitate indicates the presence of alkaloids.

Test for Tannins: FeCl₃ Test

Add 3 drops of 5% FeCl₃ to 1 mL of extract. Formation of a greenish-black precipitate indicates the presence of tannins in the extract.

Test for Flavonoids: Shibata's reaction

Warm 3 mL of extract with three pieces of Magnesium turnings and mix it with 3 drops of HCl; the appearance of pink/ orange colouration indicates the presence of flavonoids.

Test for Saponins: Frothing test

Mix 1 mL of extract and 3 mL of water in a test tube and shake the mix vigorously. Persistent frothing for a minimum of 5 minutes can be considered as an indication of the presence of saponins in the extract.

Test for Glycosides: Fehling's Test

Add 10 mL of 50% HCl to 2 mL of the extract in a test tube. Heat the mixture in a boiling water bath for 30 minutes. Add 5 mL of Fehling's solution (mixture of solution A and solution B) and boil the mixture for 5 minutes. A brick-red precipitate indicates the presence of glycosides in the extract.

Test for Cardiac glycosides: Keller-Kiliani Test

Mix 2 mL of the extract with 1 mL of

glacial acetic acid, add a few drops of FeCl_3 and conc. H_2SO_4 to it. The appearance of green-blue colour indicates the presence of cardiac glycosides in the extract.

Test for Sugars: Molisch's test

Treat 2 mL of extract solution with a few drops of 15% ethanolic alpha-naphthol solution in a test tube and then add 2 mL of concentrated H_2SO_4 along the sides of the test tube. The formation of a reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

Test for Anthraquinones: Borntrager's Test

Shake 5 mL of dried extract with 3 mL petroleum ether. Add 2 mL 25% ammonia solution to the filtrate. Red colouration can be an indication of anthraquinones in the extract.

Test for Steroids: Liebermann's Burchard Test

Add 2 mL of acetic anhydride to 0.5g of methanolic extract and then 2 mL of H_2SO_4 . The change of colour from violet to blue or green in the sample indicates the presence of steroids.

Test for Terpenoids: Salkowski Test

Shake 5 mL of extract with 2 mL of chloroform and add 2 mL of conc. H_2SO_4 along the sides of the test tube. A reddish-brown colouration at the interface can be considered as the presence of terpenoids in the extract.

Test for Amino acids: Ninhydrin test

Heat the extract with the Ninhydrin reagent for a few minutes. Development of purple colour indicates the presence of amino acids.

Antioxidant assay

DPPH Free Radical Scavenging Assay

Antioxidant activity of extracts was tested by DPPH free radical scavenging assay method.⁷ Distinct concentrations of methanolic plant extracts were mixed with 3 mL of 0.004% methanolic solution containing DPPH and incubated in darkness for 30 minutes at 37°C. After incubation, the absorbance was measured at 515 nm using UV-Visible spectrophotometer (Systronics- 116). The DPPH radical scavenging activity of extracts was calculated with reference to the absorbance of the control (mixture of methanol (5 mL) and DPPH solution (3 mL)) by using the following equation;

$$\% \text{ of Inhibition of DPPH} = [A(c) - A(s) / A(c)] \times 100$$

Where, A(c)= Absorbance of control,

A(s)= Absorbance of Sample. The DPPH free radical scavenging of standard antioxidant was also analysed by replacing the extract with Vitamin C. IC50 values were calculated as the concentration of extract or standard required to get a % inhibition of 50% by graphical method.

Nitric Oxide Scavenging Assay

Nitric oxide scavenging potential of the plant extract was determined using standard procedure.⁸ Different concentrations of methanolic plant extracts, mixed with 10 mM Phosphate buffer saline and 2 mL of 10 mM Sodium nitroprusside, were incubated for 2 hours 30 minutes at room temperature. Following incubation, 0.5 mL of the reaction mixture was transferred into fresh tubes and combined with 1 mL of Sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for completing diazotization reaction. 1 mL of 0.1% N-1-naphthylethylenediamine dihydrochloride was added to each test tube, mixed well and allowed to stand for 30 minutes and absorbance was measured at 546 nm using UV-Visible spectrophotometer (Systronics- 116). A mixture of 1 mL ethanol and 2 mL of phosphate buffer saline was considered as blank. The nitric oxide scavenging activity of extracts was calculated with reference to the absorbance of the control (without extract) by using the equation.

$$\text{Nitric oxide scavenged (\%)} = [A(\text{con}) - A(\text{test}) / A(\text{con})] \times 100$$

Where A(con)= absorbance of control, A(test)= absorbance of extract sample. Nitric oxide scavenging activity of the standard antioxidant was also evaluated by replacing the extract with Vitamin C. IC50 values were calculated as the concentration of extract or standard required to get a % inhibition of 50% by graphical method.

Phosphomolybdenum Reducing Power Assay

Antioxidant activity of plant extracts was evaluated using Phosphomolybdenum reducing power assay.⁹ Varying concentrations of plant extracts were mixed with the reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM ammonium molybdate) in 1:10 ratio. The mixture was incubated in a boiling water bath for 90 minutes. The mixture-containing tubes were allowed to cool, and absorbance was recorded

at 765 nm using UV-Visible spectrophotometer (Systronics- 116). A mixture of ethanol and reagent (1:10 ratio) was considered as control. The antioxidant activity using the phosphomolybdenum reducing power assay was also tested using standard antioxidant (Vitamin C).

Ferric reducing antioxidant power assay

The determination of reducing power based on reduction of Fe was done as per the standard procedure.¹⁰ Different concentrations of extracts were made by mixing the methanolic extracts with 0.2 M phosphate buffer (pH= 6.6). 2 mL of 1% potassium ferri-cyanide was added to each test tube, mixed well using a vortex mixture and incubated for 20 minutes at 50°C. After incubation, 2 mL of 10% trichloroacetic acid was added, and the reaction mixture was centrifuged at 3000 rpm for 10 minutes. 2 mL of supernatant was collected and mixed with 1 mL distilled water and 1 mL freshly prepared 0.1% Ferric chloride. Absorbance was recorded at 700 nm using UV-Visible spectrophotometer (Systronics- 116).

Determination of Total Phenolic content

The total phenolic content in the sample was quantified by colorimetric method using Folin-Ciocalteu reagent. 1 mL of diluted Folin-Ciocalteu reagent (in 1:1 ratio with deionized water) combined with 2 mL of diluted extract (1:1 ratio of extract and water). After 2 minutes, 1 mL of 20% Sodium carbonate was added and incubated for 30 minutes in the dark. After incubation, the

absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Systronics- 116). The reaction mixture without extract was considered as blank. A standard graph was constructed with different concentrations of gallic acid.¹¹

RESULTS

Phytochemical screening

The observations of the qualitative phytochemical analysis revealed some notable differences between the petroleum ether and methanolic leaf extracts of *R. serrata* and *R. microphylla*. The methanolic extracts showed the presence of more phytochemicals compared to those of petroleum ether extracts, because alkaloids, flavonoids, tannins, glycosides, sugars, etc, can be effectively extracted in a polar solvent like methanol.

Alkaloids were present in all the extracts, although their strong presence was observed in methanolic extracts. Tannins and flavonoids were also strongly present in the methanolic extracts of both species. The presence of terpenoids and steroids was observed in both extracts of the two plants. Cardiac glycosides and saponins were not observed in any of the extracts.

The observations of preliminary phytochemical analysis of the selected plants provide a way towards detailed and quantitative analysis of the leaf extracts, which is needed to

Table 1. Preliminary Phytochemical screening of selected plants of genus *Rotheca*

Phytochemical constituents	<i>R. serrata</i>		<i>R. microphylla</i>	
	Petroleum Ether Extract	Methanolic Extract	Petroleum Ether Extract	Methanolic Extract
Alkaloids	+	++	+	++
Tannins	-	++	-	++
Flavonoids	-	++	-	++
Saponins	-	-	-	-
Glycosides	-	+	-	+
Cardiac glycosides	-	-	-	-
Sugars	-	+	-	+
Anthraquinones	+	-	+	-
Steroids	-	+	+	+
Terpenoids	++	+	+	+
Amino acids	-	+	-	+

-: absence, +: presence, ++: strong presence

study the biological applications of the selected plants.

Anti-oxidant assay

DPPH radical scavenging assay

DPPH is a chemically stable free radical that possesses a deep violet colour. Antioxidant compounds can reduce DPPH to a yellow coloured non radical, which is called as diphenyl picryl hydrazine. When DPPH is mixed with samples having antioxidant potential, the absorbance will decrease with increasing concentration of extracts. This discoloration can be considered as the free radical scavenging ability of the extract. While calculating the percentage of inhibition, higher concentrations of antioxidants show a

higher percentage of inhibition. The results of this experiment showed the antioxidant potential of extracts of *R. serrata* and *R. microphylla* in a concentration-dependent manner (Table 2). Extracts of *R. serrata* exhibited a greater scavenging activity compared to that of *R. microphylla* (Figure 1). Both the plants exhibited a result similar to that of, Vitamin C (Standard), which showed the maximum percentage of inhibition. IC₅₀ values observed for each sample are 12.9 µg/mL for Vitamin C, and 66.1 µg/mL and 74.6 µg/mL for *R. serrata* and *R. microphylla*, respectively.

Nitric oxide scavenging assay

Nitric oxide scavenging assay is a widely adopted method to determine the antioxidant

Table 2. DPPH radical scavenging assay

Concentration (µg/mL)	% of Inhibition (Mean ± SD); n=3		
	Standard (Vit C)	<i>R. serrata</i>	<i>R. microphylla</i>
Control	0.00	0.00	0.00
T1(10)	34.31 ± 2.06	26.59 ± 1.82	28.70 ± 3.02
T2(20)	66.86 ± 1.89	32.43 ± 4.66	32.63 ± 2.81
T3(50)	73.08 ± 2.64	42.70 ± 3.82	41.19 ± 5.01
T4(100)	79.18 ± 1.02	61.93 ± 2.68	56.29 ± 3.08
T5(200)	83.03 ± 2.01	78.40 ± 1.05	64.59 ± 0.69
T6(500)	96.48 ± 0.94	82.61 ± 2.12	78.02 ± 3.06

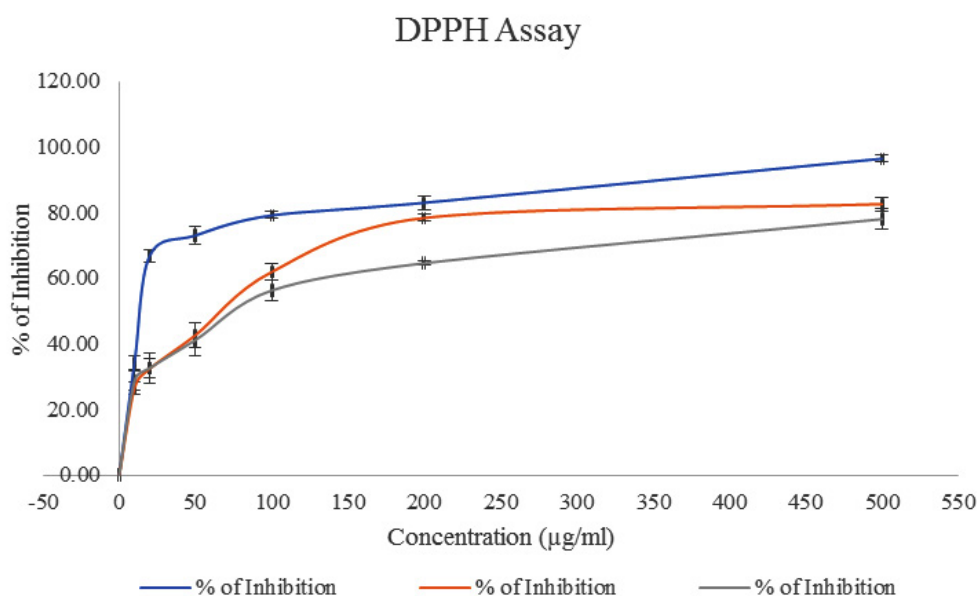


Fig. 1. DPPH radical scavenging assay

potential of plant extracts, because elevated levels of nitric oxides can result in oxidative stress and inflammation in the human body. Bioactive antioxidant chemicals in plants are capable of scavenging the nitric oxides in the body and thereby mitigating the harmful effects of it. In this experiment nitric oxide is produced using Sodium nitroprusside and it can be measured using Griess reagent using spectrophotometer in 546 nm. If the sample has the antioxidant ability the absorbance will decrease with increase the concentration of the extracts. Here in the case of *R. serrata* and *R. microphylla* there was a concentration- depended decrease in the absorbance and percentage of inhibition were given the Table 3 and results are demonstrated in the Figure 2. In all the

concentrations *R. serrata* showed more inhibitory effect than that of *R. microphylla*. The IC₅₀ values standard was determined and compared it with that of extracts. IC₅₀ value of standard was 27.8 µg/mL which was lower relative to that of *R. serrata* and *R. microphylla* i.e., 41.7 µg/mL and 57.6 µg/mL, respectively.

Phosphomolybdenum Reducing Power Assay

Phosphomolybdenum Reducing Power Assay is another method used to measure the antioxidant capacity of plant extracts. In this experiment, Mo⁶⁺ is reduced to Mo⁵⁺ due to the activity of the antioxidant property of the extracts, with the subsequent production of a green phosphate/Mo⁵⁺. The observations of this assay indicate that the leaf extracts of both plants have

Table 3. Nitric Oxide Scavenging Assay

Concentration (µg/mL)	% of Inhibition (Mean ± SD); n=3		
	Standard (Vit C)	<i>R. serrata</i>	<i>R. microphylla</i>
Control	0.00	0.00	0.00
T1(10)	20.63 ± 1.96	17.58 ± 0.87	15.80 ± 1.12
T2(20)	36.76 ± 2.05	29.52 ± 2.08	24.27 ± 2.11
T3(50)	62.23 ± 0.98	55.72 ± 1.62	49.05 ± 3.03
T4(100)	79.67 ± 2.74	73.80 ± 2.41	66.09 ± 0.81
T5(200)	84.09 ± 1.02	79.42 ± 3.26	72.75 ± 2.06
T6(500)	93.91 ± 1.97	81.82 ± 2.91	76.93 ± 2.18

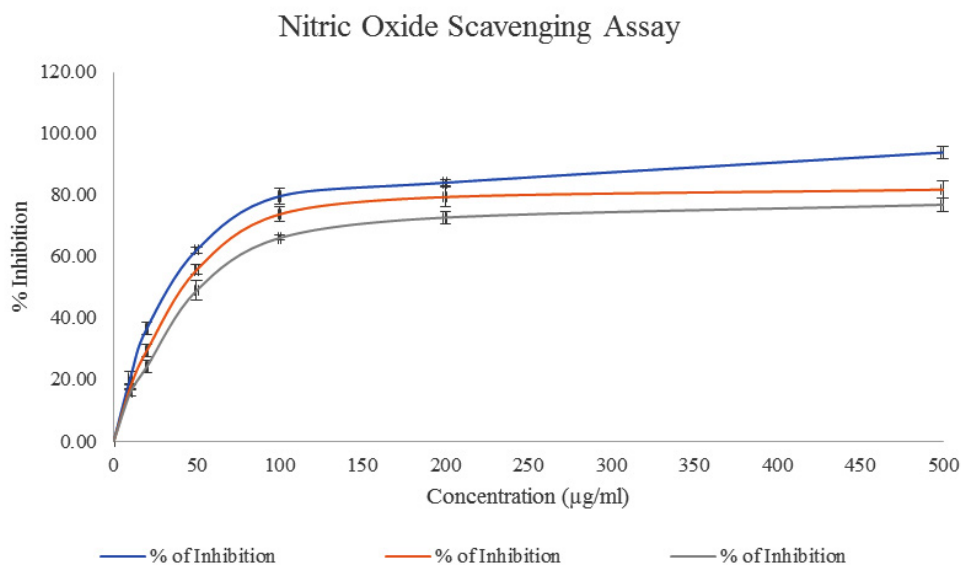


Fig. 2. Nitric oxide scavenging assay

the reducing power to convert Mo (VI) to Mo(V). and the reducing capacity shows an increase in a concentration-dependent manner (Table 4). Here, the extracts of *R. microphylla* showed greater activity in all concentrations than that of *R. serrata* (Figure 3).

Ferric reducing antioxidant power assay

FRAP assay is also a generally used procedure for determining the antioxidant ability of plant extracts. Here, the reducing power of samples to convert Fe^{3+} to Fe^{2+} is tested. If the sample has antioxidant properties, the amount of

Table 4. Phosphomolybdenum Reducing Power Assay

Concentration ($\mu\text{g/ml}$)	Standard (Vit C)	Absorbance (Mean $\pm$ SD); n=3	
		<i>R. serrata</i>	<i>R. microphylla</i>
Control	0	0	0
T1(10)	0.962 ± 0.009	0.074 ± 0.011	0.091 ± 0.004
T2(20)	0.114 ± 0.010	0.099 ± 0.006	0.101 ± 0.012
T3(50)	0.186 ± 0.012	0.147 ± 0.026	0.145 ± 0.008
T4(100)	0.363 ± 0.036	0.215 ± 0.012	0.245 ± 0.032
T5(200)	0.522 ± 0.058	0.443 ± 0.041	0.452 ± 0.016

Table 5. Ferric reducing antioxidant power assay

Concentration ($\mu\text{g/ml}$)	Standard (Vit C)	Absorbance (Mean $\pm$ SD); n=3	
		<i>R. serrata</i>	<i>R. microphylla</i>
Control	0	0	0
T1(10)	0.031 ± 0.008	0.024 ± 0.006	0.022 ± 0.009
T2(20)	0.202 ± 0.027	0.172 ± 0.104	0.160 ± 0.013
T3(50)	0.392 ± 0.061	0.366 ± 0.118	0.324 ± 0.038
T4(100)	0.816 ± 0.102	0.706 ± 0.062	0.657 ± 0.102
T5(200)	1.562 ± 0.084	1.316 ± 0.088	1.206 ± 0.027
T6 (500)	1.790 ± 0.022	1.571 ± 0.081	1.423 ± 0.052

Phosphomolybdenum Reduction Assay

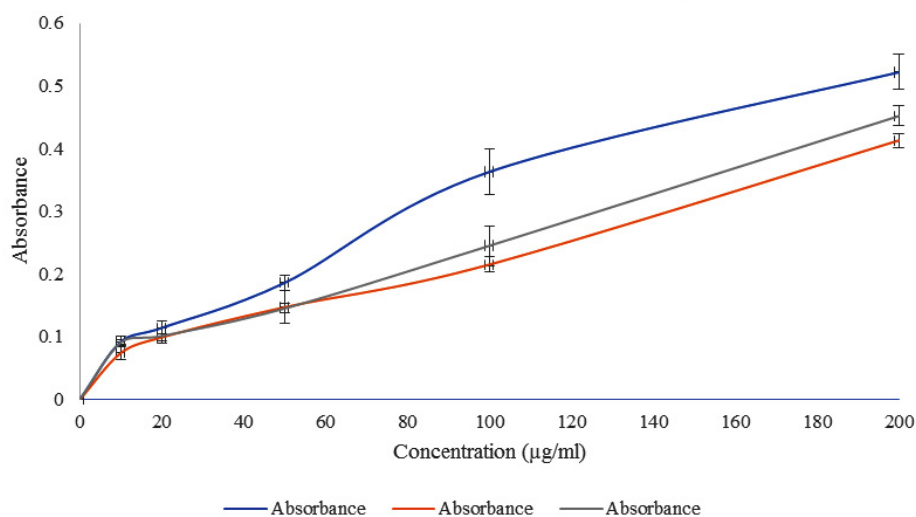


Fig. 3. Phosphomolybdenum Reducing Power Assay

Fe^{2+} will be high, and it is measured by reading the absorbance at 700 nm after mixing with FeCl_3 . In this experiment, the absorbance was directly proportional to the concentration of extracts of both plants (Table 5). From the observation, it is clear that both the selected plants have reducing power, and the extract of *R. serrata* showed more activity at all concentrations than that of *R. microphylla* (Figure 4).

Determination of Total Phenolic Content (TPC)

The TPC of the samples was quantified by the Folin-Ciocalteu reagent method, with gallic acid as the standard reference. The extract of *R. serrata* showed a TPC of 746.26 ± 8.42 μg GAE/g of extract, and that of *R. microphylla* was 661.58 ± 13.08 μg GAE/g of extract. The results indicate that both plants have a considerable concentration of phenolic compounds. This high phenolic content

can be the reason for the potential antioxidant capacities of both extracts.

Statistical analysis

All experiments were conducted in triplicate, and values were represented as mean $\pm$ standard deviation. Statistical analysis was performed using MS Excel and SPSS, and the significant values were considered as $p < 0.001$.

DISCUSSION

Natural products remain a prolific source of bioactive molecules for therapeutic development, and plant derived formulations are increasingly favoured because of their perceived safety and sustainability relative to many synthetic drugs.¹² Phenolic compounds in particular have attracted considerable interest due to their potent

Table 6. Determination of Total Phenolic Content

Concentrations ($\mu\text{g}/\text{ml}$)	Absorbance (Mean $\pm$ SD); n=3	Phenolic Content (Mean $\pm$ SD); n=3
0	0	
10	0.091 ± 0.016	
20	0.202 ± 0.021	
50	0.392 ± 0.008	
100	0.716 ± 0.101	
400	1.262 ± 0.082	
<i>R. serrata</i>	3.631 ± 0.048	746.26 ± 8.42 μg GAE/g of extract
<i>R. microphylla</i>	3.232 ± 1.161	661.58 ± 13.08 μg GAE/g of extract

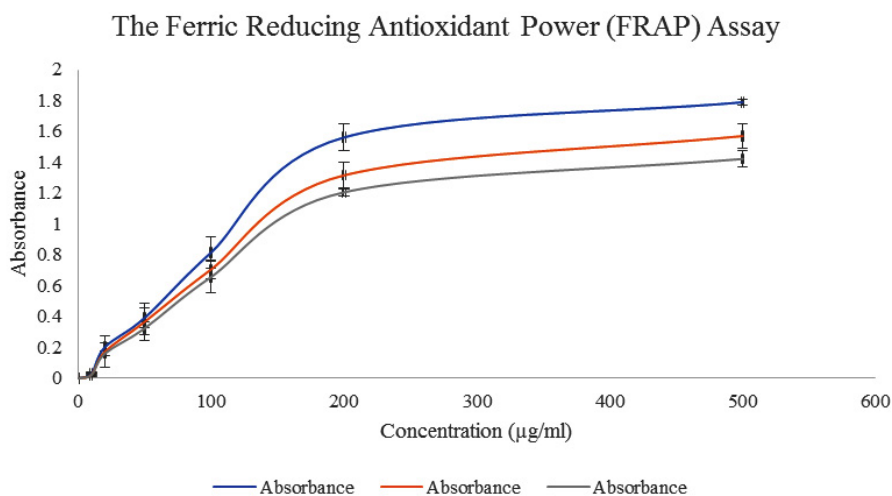


Fig. 4. Ferric reducing antioxidant power assay

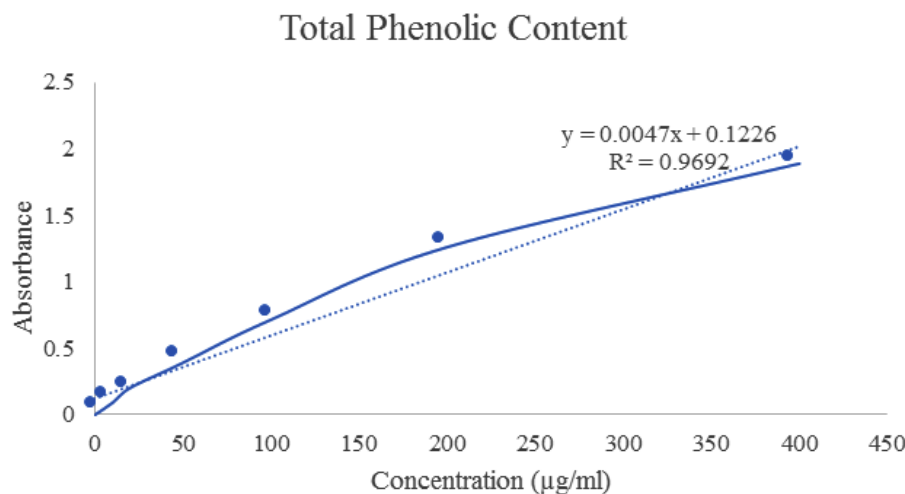


Fig. 5. Standard curve for determination of Total Phenolic Content

antioxidant properties and their documented association with reduced oxidative stress across diverse plant extracts.¹³ Previous studies have reported strong correlations between phenolic content and antioxidant capacity, underscoring the central role of phenolics in the free radical scavenging activity of botanical preparations.^{14,15}

In this study, the methanolic extracts demonstrated superior antioxidant activity compared with extracts obtained using other solvents, indicating greater extraction efficiency for antioxidant constituents in methanol. Chemical fingerprinting by GCMS and FTIR of the methanolic fraction revealed triterpenes, phenolic compounds and hydrocarbons as major constituents.⁴ These compound classes have been implicated in antiplasmodial and antiparasitic activity and are consistent with the traditional antimalarial use of *Rotheca* species. Complementary phytochemical surveys of *Rotheca serrata* have reported flavonoids, phenolics, alkaloids, glycosides and terpenoids, and recent work has confirmed significant antioxidant and anti inflammatory activities for extracts of this species.³ Antioxidant and anti inflammatory actions are mechanistically relevant because they can attenuate oxidative damage and inflammation that exacerbate parasitic and metabolic disorders, thereby enhancing the therapeutic profile of plant extracts.

Our qualitative screening identified multiple bioactive classes in leaves of *R. serrata* and *R. microphylla*, including alkaloids, tannins,

flavonoids, glycosides, terpenoids, steroids and anthraquinones. The phytochemical profile of *R. serrata* observed in the present investigation corroborates earlier reports demonstrating the presence of comparable secondary metabolites in this species.¹⁶ Such bioactive constituents have been variably linked to antioxidant, antimicrobial, anticancer, anti inflammatory and lipid lowering effects, highlighting the pharmacological properties of these species.¹⁷ Notably, prior studies on *C. serratum* (syn. *R. serrata*) also reported a dominance of polyphenolics over other phytochemical classes and attributed several pharmacological effects primarily to polyphenolic constituents or to their combined action with other metabolites.^{18,19} The absence of saponins in our methanolic leaf extracts agrees with earlier qualitative analyses of *C. serratum* roots and leaves,²⁰ that similarly failed to detect saponins, though saponins have been reported in some ethanolic extracts of *R. microphylla*.⁵ Such interstudy variation likely reflects differences in plant material, extraction solvent and analytical sensitivity.

Quantitatively, total phenolic content was higher in *R. serrata* (746.26 ± 8.42 µg GAE/mg extract) than in *R. microphylla* (661.58 ± 13.08 µg GAE/mg extract), and this difference paralleled the modestly greater antioxidant activity observed for *R. serrata* across DPPH, nitric oxide scavenging, and FRAP assays. These results corroborate the frequently observed positive relationship between phenolic concentration and antioxidant capacity

reported for many medicinal plants and support the contention that phenolics are key contributors to the redox properties measured *in vitro*.²¹⁻²³

Taken together, the phytochemical composition and antioxidant profile of the studied *Rotheca* extracts provide a plausible biochemical basis for their traditional and experimentally validated bioactivities. To advance these findings toward therapeutic application, future work should prioritize isolation and structural characterization of the active constituents, determine their individual and synergistic mechanisms and perform *in vivo* efficacy and toxicity testing alongside pharmacokinetic evaluation. Such studies will be critical for standardizing extracts and assessing their translational potential.

CONCLUSION

The present investigation demonstrated that both *R. serrata* and *R. microphylla* exhibit the presence of various groups of phytochemical compounds and considerable antioxidant potential. Qualitative phytochemical evaluation revealed that both selected plants possess comparable phytochemical composition, indicating the occurrence of similar classes of bioactive constituents. Determination of antioxidant activity with four distinct *in vitro* assays indicated notable free radical scavenging ability and reducing capacity of both plants. *R. serrata* showed comparatively higher total phenolic content (TPC) and antioxidant activity in most of the tests performed. *R. microphylla* also showed antioxidant potential, whereas its activity was mildly lower than that of *R. serrata*, except for the phosphomolybdenum reducing power assay, indicating variation in the concentration or mechanism of action of specific antioxidant constituents of the plants.

Overall, the observations support that both selected species can be considered as a potential source of antioxidants with significant medicinal and nutraceutical importance. To advance these findings toward therapeutic application, future work should prioritize isolation and structural characterization of the active constituents, determine their individual and synergistic mechanisms and perform *in vivo* efficacy and toxicity testing alongside pharmacokinetic evaluation. Such

studies will be critical for standardizing extracts and assessing their translational potential.

ACKNOWLEDGEMENT

The authors acknowledge the constant support and encouragement by the principal CMS College Kottayam (Autonomous) and B.C.M. College Kottayam (Autonomous). We would like to express our special thanks to Doctor John's Biotech Centre for Research and Development for providing technical assistance and instrumental facilities for the successful completion of this work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Antu Mariya Jose: Conceptualization, Data Collection, Analysis, Methodology, Writing – Original Draft; Jinu John: Editing, Resources; Rogimon Plammoottil Thomas: Visualization, Supervision, Project Administration.

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