

Bioactive Compounds Profiling and Assessment of *In Vitro* Antioxidant Activity of *Ruellia prostrata* Leaf Extract

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ABSTRACT

Plants feature a range of biochemical processes and drug-like qualities due to their chemical makeup. In actuality, traditional medicine has been actively used throughout the world to treat basic medical requirements as well as to prevent and manage chronic diseases linked to particular lifestyles. Traditional medicine primarily uses a variety of medicinal plants and herbal preparations as medicines. The current study's objective was to assess leaf extracts' phytochemical composition using GC/MS analysis. The hydro-ethanolic solvent, water, ethanol, hexane, and petroleum ether were among the solvents used for the qualitative examination of the leaf extracts. The hydro-ethanolic extract was selected for additional research because it contains much more phytochemicals. The hydro-ethanolic extract of *Ruellia prostrata* leaves included significant levels of saponins (110.41 ± 7.72), tannins (80.42 ± 5.62), steroids (97.42 ± 6.81), phenols (207.38 ± 14.51), and flavonoids (176.52 ± 12.35). Hexadecanoic acid ethyl ester, octadecanoic acid, tetradecane, n2-hexadecen-1-ol (3, 7, 11, 15-tetramethyl), phytol acetate, and products of its ethyl ester were the main chemicals found in the GC-MS experiment on the leaf extract. Furthermore, the HPLC analysis verified that the hydro-ethanolic extract included numerous bioactive compounds, including quercetin, epicatechin, kaempferol, rutin, hyperoside, luteolin, and caffeic acid. Ascorbic acid was used as the standard reference compound in a number of tests to assess the extract's antioxidant capability, including nitric oxide scavenging, hydrogen peroxide, DPPH, and superoxide scavenging activity at different doses. As a scavenger of DPPH radicals, hydrogen peroxide, and superoxide, the leaf extract was shown to exhibit IC₅₀ values of 34.23, 50.40, and 45.76 µg/ml, respectively. According to the study's findings, *Ruellia prostrata* leaf extract contains phytonutrients and may have antioxidant properties.

Keywords: Phytochemicals, Analysis of Quantitative and Qualitative, *Ruellia prostrata*, GCMS and antioxidants.

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INTRODUCTION

The field of phytochemical research is rapidly becoming popular in the business and scientific world. The impacts of most plant chemicals and pigments on both people and animals are now well known. The health effects of these chemicals are huge, and are currently being well studied and comprehended at a fundamental level. The therapeutic substances of the natural resources have been used as the source of therapeutic substances thousands of years ago and several recent medicines are based on the natural resources (Deepak Bharati and Abhijeet Puri, 2023). Traditional treatments are a major source of novel compounds that may be utilized in the creation of chemotherapy treatment medications. While many others

are yet unknown, the most significant applications and advantages of several crops have been identified and enumerated. Research in pharmaceutical and pharmacological should be performed to determine their therapeutic potential and explore their uses (Mavie Rose, 2024). Hartati et al. (2024) argue that the use of plants as a taxonomic marker by modern medicine to produce more complex semi-synthetic chemical compounds, a model of new synthetic chemicals, and a source of direct medicinal chemicals.

One of the main goals of natural medicinal chemistry research is the extraction and isolation of natural medications. The initial step to acquiring the appropriate

active ingredients is to extract and isolate natural drugs that have been clinically proven to be active or have a biological activity determined by screening of activities (Takahashi et al., 2014). It is thought that because of their antioxidant properties, phytochemicals can either prevent or cure disease (Halliwell and Gutteridge, 1992). *In vivo*, antioxidants shield other molecules from oxidation when they come into contact with reactive oxygen species and free radicals, which have been linked to the development of several diseases (Hussen and Endalew, 2023). antioxidants, both synthetic and natural, that can stop oxidation and scavenge free radicals (Hayat et al., 2010). Because of the fact that synthetic antioxidants are not easily soluble and could lead to liver damage, cancer and other complications during the industrial processing process, use of synthetic antioxidants is currently being limited (Barlow, 1990). The trend is increasingly towards the use of natural antioxidants made by plants (Hussen and Endalew, 2023). *Ruellia prostrata* was chosen due to its excellent, scientifically confirmed antibacterial, anti-inflammatory and antioxidant characteristics that are substantiated by ancient experience in traditional medicine.

Ruellia prostrata is a valuable therapeutic herb anti-inflammatory, diuretic, anti-diabetic, analgesic (pain reliever) in fevers and wounds, urinary and gonorrhea problems. Additionally, the plant is used in teas and poultices to cure a variety of illnesses, including spermatorrhea, skin conditions, and as an antioxidant. Scientific research on it has been found to be inadequate, and there is a severe lack of characterization of the main bioactive chemicals. In contrast, this work used the NO, DPPH, H₂O₂, and SO tests as well as qualitative and quantitative phytochemical analysis utilizing different solvents, GCMS, and HPLC to assess the antioxidant activity of leaf *Ruellia prostrata* extract

MATERIALS AND METHODS

Plant materials Collection

The specimen (*Ruellia prostrata*) was picked in Sengipatti Village of Thanjavur District, Tamil Nadu, India in December 2023. *Ruellia prostrata* leaves were completely moistened and dusted off. The leaves having been roughly cut, were left to dry in three weeks. The leaves were pulverized. The leaves that were powdered were stored in a tightly sealed container and utilized for a variety of analyses in phytochemical.

Preparation of plant extracts.

Ten grams of crushed *Ruellia prostrata* leaves were extracted. They were extracted over the course of a day using intermittent shaking and cold extraction (maceration method) in a variety of solvents, including aqueous, chloroform, ethanol, hexane, and hydro-ethanolic (ethanol and water (70:30)). The extracts were subsequently filtered via Whatman filter paper No. 1, and additional phytochemical analytical research was conducted on the filtrate..

Analysis of Phytochemical

The preliminary phytochemical screening was done using the standard protocol, as developed by Harborne (1973, 1984), Mavie Rose et al. (2024), Trease and Evans (1989), and Sofowara (1993). *Ruellia prostrata* leaves' total phenolic content has been measured using a slightly modified version of Kim et al. (2003)'s spectrometer approach. According to Katasani (2011), total flavonoids test was conducted. The total amount of flavonoid was determined by the colorimetric process of chloride of aluminum. The Bajaj and Devsharma (1977) procedure was followed for doing the total tannins test. Colorimetric methods were used to determine the overall saponin level of the leaf materials of *Ruellia prostrata* (Hiai et al., 1976). The technique established by Attarde Daksha et al. (2010) was used to find out the steroids.

GC-MS Analysis

The following settings were used for GC-MS analysis (on a Shimadzu Corporation 2010 plus that included an AOC-20i auto sampling device) and gas liquid chromatograph coupled with mass spectrometer instrument: column RTX 5Ms (column diameter is 0.32 mm, column length is 30 m, column width is 0.50 μ m), which was operated in electron impact mode at 70 eV; carrier gas was helium gas (99.99%), injection volume was 5 μ L, and the constant flow rate was 1.73 ml/min. I was run at a split ratio of 10:1, with an injector temperature of 270 °C and an ion source temperature of 200 °C. After adjusting the oven's temperature to 40 degrees Celsius in an isothermal condition for two minutes, it was raised by 8 degrees Celsius per minute until it reached 150 degrees, at which point it was set to 280 degrees. Mass spectra were collected at 70 eV using components ranging from 40 to 450 Da and a scan time of 0.5 seconds. The GC takes 51.25 minutes on average (Srinivasan et al., 2013). By comparing each component's average peak area to the total areas, the relative percent quantity of each component was calculated. The National Institute of Standards and Technology ("NIST") provided assistance in interpreting the GC-MS data, which included more than 62,000 patterns. The spectra of the known components from the NIST collection were compared to the spectrum of the unknown component. The test compounds' names, molecular weights, and structural components were determined (Dukes, 2013).

Analysis of HPLC

The fractions of flavonoids were analyzed using HPLC process (Paranthaman et al., 2012; Weerasak Samee et al., 2007). *Ruellia prostrata* leaf extract was subjected to HPLC analysis using chromatography equipment (Shimadzu Class-VPV6.14SP2, Japan), which included a visible ultraviolet detector and an automated sampling system with a fixed loop of 20 μ L. The purity of molecules was significantly affected by the gradient elution of solvents A (25:1 v/v water-acetic acid) and B (methanol). Using a dual pump system, the ratio of solvent A (water-acetic acid 25:1, v/v) to solvent B (methanol) was changed

to create the solvent gradients. After 4 minutes, solvent B was put in 50 percent and then 80 percent after 10 minutes. The samples were then run in a UV detector (Lamp-D2), which detected the samples at 280 nm. The chromatographic information was all measured and analyzed with Autochro software.

Activity Of anti oxidants in vitro.

Shimada et al. (1992) also established the DPPH radical-scavenging activity in their approach in their methodology. The scavenging activity of radicals of superoxide anions was determined by using the Liu et al. (1997) approach. The Garrat (1964) method was used to measure the scavenging ability of nitric oxide radicals. Radon scavengers of Hydrogen peroxide (Ruch et al., 1989).

Statistical analysis

Three duplicates of each test were conducted. The number of samples needed to reduce the concentration of the free radicals by 50% or IC50 was estimated using the linear regression technique with the graphpad Instat (version 3) software, which is based on Microsoft windows. Mean $\pm$ standard deviation was used to graphically represent the results.

RESULTS AND DISCUSSION

Phytochemicals qualitative analysis

In the phytochemical research of bioactive compounds in plant materials, proper choice of extraction method is

crucial. *Ruellia prostrata* is an important medicinal plant in the conventional systems. Its application is as an anti-inflammatory, diuretic, anti-diabetic and analgesic (pain reliever) in fevers, wounds and urinary/gonorrhea problems. Part of the plant including roots and seeds is used as teas and poultice, to treat spermatorrhea, skin conditions and it is used as an antioxidant against diseases. In contrast to quantitative analysis, which determines the presence of chemicals, colorimetric tests are used in quality phytochemical analysis to determine the presence of compounds. Two beneficial chromatography methods that aid in separation, identification and quantification of the substances within the complex mixtures are the GC-MS and the HPLC. Alam et al. (2021) and Xu et al. (2020) reported similar results for *Ruellia* species' GCMS and HPLC. The first procedure in all the studies conducted on the medicinal plants is extraction and it greatly influences the results of the study. This study's research problem is to examine the qualitative analysis of several leaf extracts from *Ruellia prostrata*. The yield of each extract is displayed in Table 1. The yields of petroleum, ethanol, hexane, hydro-ethanol, and aqueous extracts were 9.16%, 13.14%, 6.02%, 16.37%, and 5.32%, respectively. Compounds including steroids, saponin, anthroquinone, flavonoids, coumarins, terpenoids, emodins, triterpenoids, alkaloids, polyphenol, tannin, glycoside, and anthocyanins were found in the aqueous extract of *Ruellia prostrata* leaves, according to a qualitative study.

Table 1: The yield of diverse extracts of *Ruellia prostrata* leaf

Extracts	Crude extract weight (W1) in grams	Weight (g) of the (W2) sample obtained	The extract's yield (s) (%)
Aqueous	00.916	10	9.16
Ethanol	01.314	10	13.14
Hexane	00.602	10	6.02
Hydro-ethanol	01.637	10	16.37
Petroleum ether	00.532	10	5.32

The chloroform extract of *Ruellia prostrata* leaves was found to be devoid of anthocyanins, glycoside, alkaloids, emodins, coumarins, tannin, steroids, terpenoids, anthroquinone, triterpenoids, and saponin. When compared to tannin, flavonoids, emodins, anthroquinone, alkaloids, anthocyanins, and an extract of *Ruellia prostrata* leaves in hexane, the presence of saponin, steroids, terpenoids, polyphenol, triterpenoids, glycoside, and coumarins was found (Table 2). The *Ruellia prostrata* leaf hydro-ethanolic

extract will be used for more investigation because it has more phytochemicals than the other extracts. *Ruellia prostrata* leaf hydro-ethanolic extract's phenol, tannin, steroid saponin, and flavonoid, content were quantitatively analyzed in Table 3. The leaf extract of *Ruellia prostrata* was determined to have the largest amount of phenol and the least amount of tannin.

Table 2: Phytochemicals qualitative analysis of *Ruellia prostrata* leaf

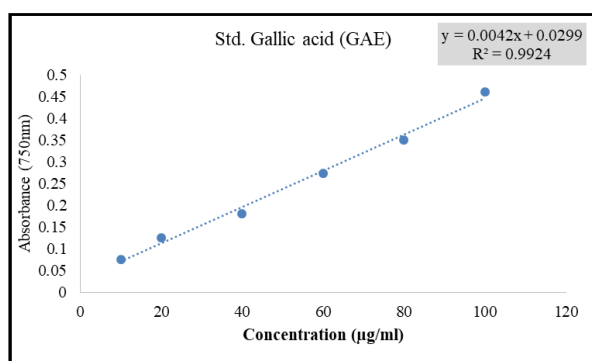
S. No	Phytochemicals	Extracts
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		Aqueous	Ethanol	Hydro-ethanolic	Chloroform	Hexane
1	Coumarins	+	++	++	--	+
2	Saponin	++	++	++	+	+
3	Polyphenol	++	++	++	+	+
4	Alkaloids	+	+	+	--	--
5	Terpenoids	+	++	++	+	+
6	Steroids	++	++	++	+	+
7	Triterpenoids	++	+	++	+	+
8	Anthroquinone	+	+	++	+	--
9	Flavonoids	++	++	++	+	--
10	Tannin	++	+	++	--	--
11	Emodins	+	+	+	--	--
12	Anthocyanins	+	--	--	--	--
13	Glycoside	+	+	++	--	+

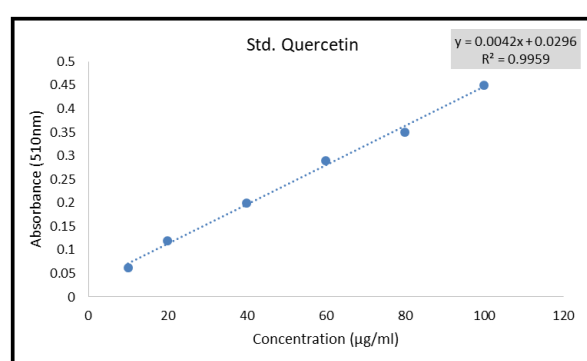
(+) Existing, (--) Non-Existing, and (++) High concentration (High intensity of colour)

Table 3: Hydro-ethanolic extract of *Ruellia prostrata* leaf: quantitative investigation of tannin, saponin, steroid, flavonoid, and phenol content

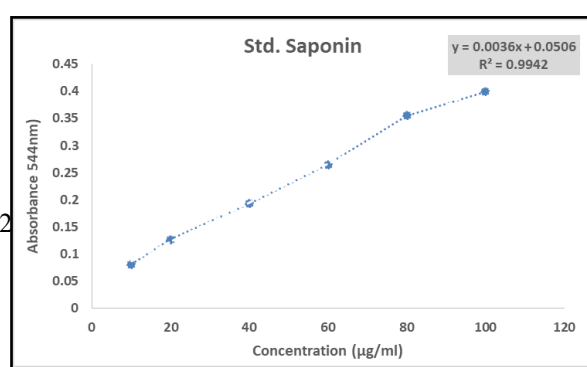
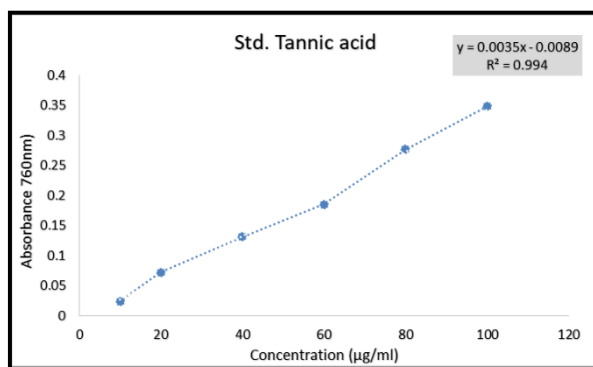
Name of Extract	Total phenol (Expressed in milligrams of gallic acid equivalents, or GAE) per gram	Flavonoid (Equivalents of quercetin in milligrams per gram)	Tannin (Tannic acid equivalents in milligrams per gram)	Saponin (Quillaja equivalents in milligrams per gram)	Steroid (Equivalents of chlosterol in milligrams per gram)
Hydro-ethanolic	207.38 ± 14.51	176.52 ± 12.35	80.42 ± 5.62	110.41 ± 7.72	97.42 ± 6.81



(a)

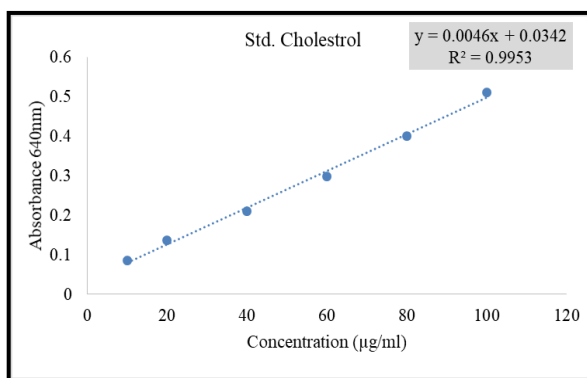


(b)



(c)

(d)



(e)

Figure 1 The typical curve for Phenol using (a) Gallic acid (b) Quercetin (c) Tannic acid, (d) Saponin and (e) cholesterol

Screening of bioactive substances in *Ruellia prostrata* by GC MS analysis

This paper involved the hydroethanol extract of *Ruellia prostrata* leaf in order to determine 30 chemical compounds in the extract using gas chromatography-mass spectroscopic analysis. The identified components' spectra, which were kept in the NIST library, were compared to the spectrum of the unknown component. Table 3 lists the molecular formula and weight of the active ingredients, retention time and concentration of the active ingredients.

The results of this study have christened 30 substances with different biological properties. Table 4 shows the *Ruellia prostrata* leaf extract's biological properties (Dr. Dukes, 2013). Tetradecane, phytol acetate, octadecanoic acid, ethyl ester, n-hexadecanoic acid, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl were the most common chemicals found.

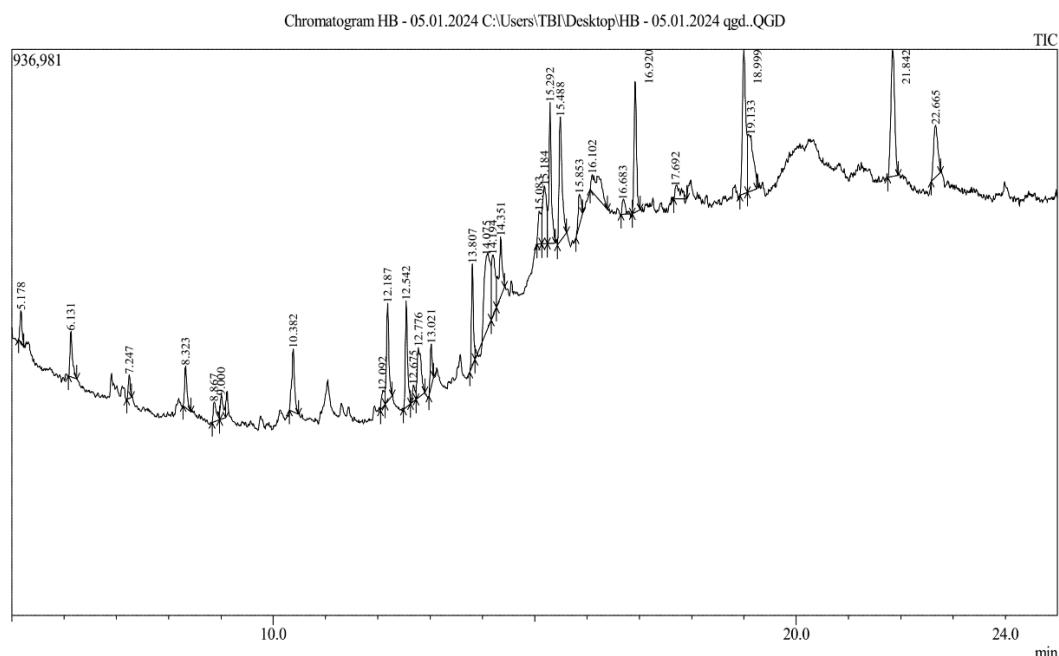

 Figure 5: Chromatogram of GC-MS of *Ruellia prostrata* leaf extract

 Table 3: Detection of bioactivity compound in *Ruellia prostrata* leaf extract using GC-MS techniques

Peak #	R. Time	Area %	Height %	A/H	M. Weight (g/mol)	M. Formula	Name of the isolated compound
1	5.178	1.09	1.76	2.66	444	C ₁₂ H ₃₆ O ₆ Si ₆	Cyclohexasiloxane, dodecamethyl-
2	6.131	1.90	2.50	3.26	104	C ₄ H ₈ O ₃	2-Methoxy-1,3-dioxolane
3	7.247	0.77	1.30	2.52	198	C ₁₄ H ₃₀	Tetradecane
4	8.323	1.69	2.31	3.14	652	C ₁₆ H ₄₈ O ₁₀ Si ₉	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane
5	8.867	1.18	1.08	4.68	206	C ₁₄ H ₂₂ O	2,6-Ditert-butylphenol
6	9.000	1.33	1.27	4.46	532	C ₁₆ H ₄₈ O ₆ Si ₇	1,1,1,5,7,7,7-Heptamethyl-3,3,5-tris(trimethylsiloxy)tetrasiloxane
7	10.382	3.00	3.46	3.73	472	C ₂₀ H ₄₀ O ₅ Si ₄	Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester
8	12.092	0.99	0.89	4.73	157	C ₈ H ₁₅ NO ₂	Oxiranecarboxamide, 2-ethyl-3-propyl-
9	12.187	3.99	5.40	3.17	472	C ₂₀ H ₄₀ O ₅ Si ₄	3,4-Dihydroxymandelic acid-tetratms
10	12.542	4.10	5.85	3.00	278	C ₂₀ H ₃₈	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
11	12.675	0.82	0.91	3.87	268	C ₁₈ H ₃₆ O	2-Pentadecanone, 6,10,14-trimethyl-
12	12.776	3.31	2.75	5.16	399	C ₁₈ H ₃₇ NO ₃ Si ₃	Phenethylamine, N-methyl-.beta.,3,4-tris(trimethylsiloxy)-
13	13.021	1.48	2.33	2.72	338	C ₂₂ H ₄₂ O ₂	Phytol, acetate
14	13.807	3.32	5.61	2.54	576	C ₁₈ H ₅₂ O ₇ Si ₇	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
15	14.075	7.84	4.19	8.02	256	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
16	14.194	3.79	3.38	4.80	312	C ₂₀ H ₄₀ O ₂	Octadecanoic acid, ethyl ester
17	14.351	3.50	3.29	4.56	481	C ₁₉ H ₃₄ F ₃ NO ₄ Si ₃	N-(Trifluoroacetyl)-O,O',O''-tris(trimethylsilyl)norepinephrine
18	15.083	1.88	1.80	4.47	578	C ₁₆ H ₅₀ O ₇ Si ₈	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
19	15.184	3.84	3.18	5.18	228	C ₁₅ H ₃₂ O	n-Pentadecanol

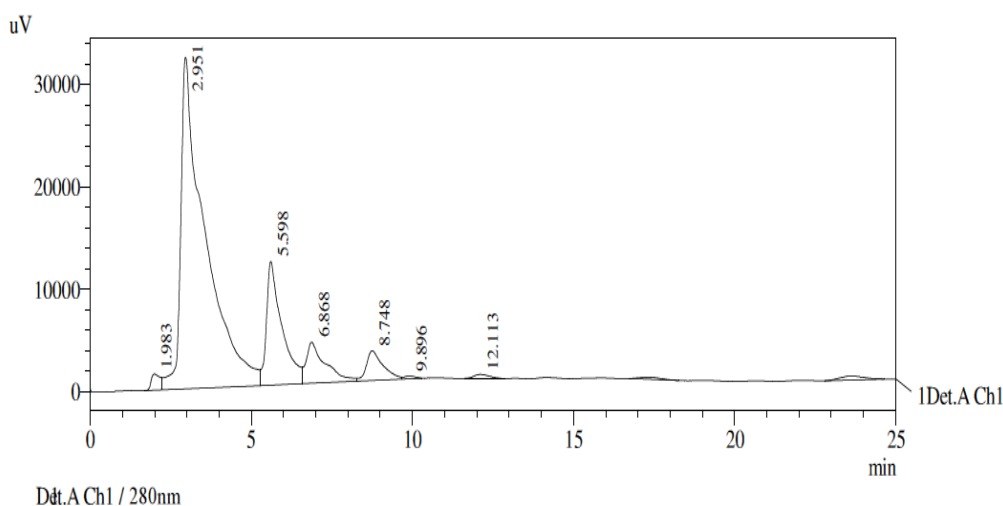
20	15.292	5.68	7.76	3.14	472	C ₂₀ H ₄₀ O ₅ Si ₄	3,4-Dihydroxymandelic acid-tetratms
21	15.488	6.45	6.84	4.05	296	C ₂₀ H ₄₀ O	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl
22	15.853	1.64	1.74	4.04	156	C ₁₀ H ₂₀ O	D-Neoisomenthol
23	16.102	3.44	0.96	15.41	310	C ₁₉ H ₃₄ O ₃	Methoprene
24	16.683	0.76	0.75	4.33	356	C ₁₆ H ₃₂ O ₃ Si ₃	2,5-Dihydroxybenzyl alcohol 3tms
25	16.920	5.40	7.21	3.21	666	C ₁₈ H ₅₄ O ₉ Si ₉	Octadecamethyl-cyclononasiloxane
26	17.692	1.20	0.66	7.82	458	C ₁₄ H ₄₂ O ₅ Si ₆	Hexasiloxane, tetradecamethyl-
27	18.999	8.45	7.94	4.56	740	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane
28	19.133	5.29	3.07	7.39	243	C ₁₂ H ₁₀ FN ₅	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
29	21.842	8.17	6.95	5.04	384	C ₁₇ H ₃₂ O ₄ Si ₃	Benzeneacetic acid, 2,5-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester
30	22.665	3.70	2.85	5.56	390	C ₂₄ H ₃₈ O ₄	Di-n-octyl phthalate

Table 4: Biological activity of bioactive compounds screened by GC-MS

R. Time	Name of molecule	Biological action
7.247	Tetradecane	Antimicrobial activity
13.021	Phytol acetate	Hepatoprotective, anti-androgenic, anti-inflammatory, anti-cancer, anti-coronary, anti-arthritis, and nematocide
14.075	n-Hexadecanoic acid	Five-Alpha reductase inhibitor, antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic, and hemolytic.
14.194	Octadecanoic acid, ethyl ester	Dermatitogenic, cancer-preventive, antiandrogenic, and anti-inflammatory, properties
15.488	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	Diuretic, anti-inflammatory, antimicrobial, and cancer preventative. Antioxidant

**Source: Dr. Duke's phytochemical and ethnobotanical databases

HPLC Analysis

Fig 6: HPLC profile of Plant extract at 280nm

Table 5: Profile of HPLC in Plant extract at 280nm

Peak #	Ret. Time	Area (Milli-area-seconds, or mAs)	Elevation in microvolts (μV)	Area (%)	Height (%)	Chemicals Compounds recognized by literature (Tapan Seal, 2016, Baram Ahmed, 2013, Weerasak, 2007, Xu <i>et al.</i> , 2020, Paranthaman, 2012)
1	1.983	31328	1581	1.274	2.919	Kaempferol
2	2.951	1702045	32372	69.232	59.765	Quercetin
3	5.598	403122	12059	16.397	22.263	Luteolin
4	6.868	167206	4012	6.801	7.407	Rutin
5	8.748	105329	2922	4.284	5.394	Hyperoside
6	9.896	6965	263	1.107	1.159	Caffeic acid
7	12.113	12415	384	0.905	1.093	Epicatechin
Total		2428411	53592	100.000	100.000	

Fig. 6 shows the result of chromatograms of flavonoid components in the *Ruellia prostrata* leaf extracts at 280 nm wavelength. The highest retention times of each of the flavonoid molecules at each wavelength are shown in Table 5. This chemical is a flavonoid chemical found in the *Ruellia prostrata* leaf extract by relating the peaks of chromatographic to the retention time (Rt) as compared to previous studies. The hydro-ethanolic extract of *Ruellia prostrata* leaves included epicatechin, rutin, kaempferol, luteolin, quercetin, caffeine, and hyperoside, according to HPLC analysis. The compounds found in the corresponding groupings are responsible for the plants' diverse therapeutic qualities. The presence of water soluble antioxidants called flavonoids that possess anticancer and free radical scavenging effects contributes to the antioxidant properties of plants (Yadav and Agarwala, 2011). The alkaloids were already known to have analgesic and antibacterial properties (Nassar *et al.*, 2010), and the terpenoid were well known to have anti-inflammatory, antibacterial and anticancer properties (Chung *et al.*, 1998). Radhika *et al.* (2013) claim that tannins have antibacterial and anti-cancer qualities. By modifying glucose metabolism, which raises insulin release, triterpenoids may be able to prevent diabetes (Sabbah *et al.*, 2017). It was found that in addition to their insecticidal and antibacterial properties, steroids have cardio-tonic effects (Bagrov *et al.*, 2009). Phytosterol and phenolic compounds have been known to have an antibacterial, antioxidant, antiallergic, antimutagenic, anti-inflammatory, antidiabetic, and anti-carcinogenic properties of plants (Khan *et al.*, 2015).

Saponin content of the plant also plays a key role in the treatment of hypoglycemia and hypocholesterolemia since they aid in weight reduction and have anticancer, antibacterial, antioxidant, and antifungal effects (Lira *et al.*, 2017). Glycosides have anticancer and anticoagulant qualities, according to Xiao (2017). Ntie-Kang *et al.* (2014) attribute the anti-plasmodial and antimalarial properties to coumarins. Plants containing anthocyanin have anti-inflammatory, anti-obesity, anti-cancer and neuroprotective effects (Chien *et al.*, 2015). Rastogi *et al.* (2015) assert that plant-based anthraquinones can control the immune system and that they can be utilized in the treatment of

autoimmune diabetes. Grancellin *et al.* (2013) state that triterpenoids have anti-cancer properties. This bioactivity has a root in numerous antioxidant-containing phytochemicals found in medicinal plants. Qualitative phytochemical profiling exhibited that the antioxidant phytochemicals that were present in the plant extracts under investigation were flavonoids, phenols, and tannins.

In vitro antioxidant activity

Numerous diseases' pathophysiology and development are significantly influenced by oxidative stress (Adwas *et al.*, 2019). A single assay technique is not suitable in assessing the antioxidant potential of endogenous compounds. The current study has assessed the antioxidant properties using a diversity of antioxidant models such as DPPH, superoxide, hydrogen peroxide and nitrogen oxide scavenging activities of *Ruellia prostrata* extract.

The reason being that the DPPH method is one of the most vigorously used methods of assessing the antioxidant capacity of a plant extract. Soare *et al.* (1997) identified DPPH as an unstable free radical, which is typically used to determine the antioxidant or free radical scavenging action because of the ready absorption of antioxidant chemical by the hydrogen atoms. The interactions between antioxidants and DPPH are primarily due to the possibility of antioxidants to give up hydrogen, which will neutralize the radical (Baumann, 1979). Eradication of the free radicals is important in averting the cell damage that comes with an extensive range of diseases, including cancer. The DPPH test is a commonly used and trustworthy technique for assessing plant extracts' antioxidant potential. In this experiment, the deep violet DPPH solution is used together with the *Ruellia prostrata* leaf extract which forms diphenylpicryl hydrazine. The magnitude of this change of color varies with the concentration of the extract. The technique is prevalent in the estimation of the antioxidant activity based on its cost efficiency and speed. The capacity of *Ruellia prostrata* leaf extract to scavenge DPPH radicals was compared to ascorbic acid, as indicated in Table 6. Its scavenging action was enhanced in a

concentration-dependent manner by the hydro-ethanolic regular ascorbic acid was 38.20 µg/ml. extract. The extract's value was 34.23 µg/ml, while that of

Table 6: DPPH radical scavenging activity of hydroethanol extract of *Ruellia prostrata* leaf and compared with standard as ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Std. (Ascorbic acid)	Hydro Ethanol
20	34.07±2.38	42.38±2.96
40	51.52±3.60	50.13±3.50
60	70.36±4.92	68.14±4.76
80	82.54±5.77	80.88±5.66
100	96.95±6.78	91.13±6.37
IC ₅₀	38.20	34.23

Values in triplicate are shown as Mean ± SD.

Mammalian cells produce nitric oxide (NO), a free radical that is essential for controlling a number of physiological functions. However, excessive production of nitric oxide has been associated with a number of pathological conditions such as ulcerative colitis, multiple sclerosis, juvenile diabetes, arthritis and certain types of cancer. This has resulted in the creation of substances that might suppress overproduction of nitric oxide as a crucial field of study in addressing chronic inflammatory diseases (Hazra et al., 2008). Without effective endogenous enzyme pathways, the body uses a number of substances that are naturally found including glutathione, melatonin and dietary antioxidants as counteracting agents to the effects of nitric oxide (Garraff, 2012). Nitric oxide, a physiologically relevant chemical, is used in a number of

physiological processes (Marcocci et al., 1994). Sodium nitroprusside, which naturally creates NO in the aqueous solution at physiological pH, can be used to create nitric oxide under experimental settings. A Griess test can be used to assess how many nitrite ions are produced if the released nitric oxide reacts with oxygen. Nitric oxide scavengers compete with oxygen to lower nitric oxide levels by reducing nitrite synthesis (Marcocci et al., 1994). Table 7 displayed the *Ruellia prostrata* leaf extract's nitric oxide scavenging activity and contrasted it with that of regular ascorbic acid. The *Ruellia prostrata* leaf hydroethanolic extract's ability to scavenge nitric oxide increases with concentration. The values of the *Ruellia prostrata* IC₅₀ leaf extract and standard ascorbic acid were 45.50µg/ml and 50.40µg/ml, respectively.

Table 7: *Ruellia prostrata* leaf hydroethanol extract's capacity to scavenge nitric oxide and compare it to ascorbic acid as a reference

Concentrations (µg/ml)	% of inhibitions	
	Std. (Ascorbic acid)	Hydro Ethanol
20	26.98±1.88	20.23±1.41
40	43.65±3.05	38.88±2.72
60	68.25±4.77	65.87±4.61
80	79.76±5.58	78.57±5.49
100	97.61±6.83	88.88±6.22
IC ₅₀	45.05	50.40

Mean ± SD is displayed for values in triplicate.

Table 8: Super oxide scavenging activity of *Ruellia prostrata* leaf hydroethanol extract and ascorbic acid standard comparison

Concentrations (µg/ml)	% of inhibitions	
	Std. (Ascorbic acid)	Hydro Ethanol

	acid)	
20	24.29±1.70	25.7±1.79
40	57.74±4.04	41.9±2.93
60	65.49±4.58	66.54±4.65
80	81.69±5.71	78.52±5.49
100	96.47±6.75	91.54±6.40
IC ₅₀	42.01	47.11

Mean ± SD is exhibited for values in triplicate.

Table 9: Hydrogen peroxide (H₂O₂) scavenging activity of hydroethanol extract of *Ruellia prostrata* leaf and likened with standard as ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Std. (Ascorbic acid)	Hydro Ethanol
20	46.42±3.24	27.67±1.93
40	50±3.50	44.64±3.12
60	63.39±4.43	63.39±4.43
80	81.25±5.68	79.91±5.59
100	97.76±6.84	94.64±6.62
IC ₅₀	33.50	45.76

Mean ± SD is showed for values in triplicate.

Radical scavenging ability of the fractions was determined under NBTNADH-PMS system. The observed reduction in optical density as compared to the control condition suggests that there are bioactive molecules capable of scavenging superoxide radicals. A reduced form of molecular oxygen, the superoxide anion, is an important factor in the initiation of oxidative processes related to ageing (Rossi et al., 2024). It also participates in the production of other reactive oxygen species like hydrogen peroxide, singlet oxygen and hydroxyl radicals, which may lead to oxidative damage in biomolecules such as proteins, lipids and DNA (Todorov et al., 2023). Moreover, the superoxide anion is an oxygen-centered radical that is selectively reactive. It can be synthesized both by enzymatic systems and non-enzymatic electron transfer reactions, in which univalent reduction of molecular oxygen occurs. Furthermore, it can eliminate metal complexes, such as cytochrome c (Yu et al., 2023). The higher superoxide scavenging activity was favorably linked with the extract content in the hydroethanolic extract of *Ruellia prostrata* leaves. According to Table 8, the IC₅₀ values for the leaf extract and the standard antioxidant ascorbic acid were 47.11 µg/mL and 42.01 µg/mL, respectively. Numerous habitats, including food systems, plants, microbes, water, air, and even the human body, frequently contain trace levels of hydrogen peroxide. It can easily break down to produce highly reactive

hydroxyl radicals (•OH), which is able to cause damage to DNA and lipid peroxidation, as well as the production of oxygen (O₂) and water (H₂O). The ascorbic acid was tested and compared to the scavenging activity of the hydrogen peroxide of the extract of *Ruellia prostrata* (Table 9). The results showed a concentration effect in increasing the scavenging activity where the higher the concentration of extracts, the more the hydrogen peroxide was neutralized. The determination of the IC₅₀ values was done to be 45.76 µg/ml of the hydroethanolic leaf extract of *Ruellia prostrata* and 33.50 µg/ml of ascorbic acid. The similar antioxidant properties of plant extracts have been widely reported in previous studies, such as Craciunescu et al. (2024), Mihai et al. (2024), and Negreanu et al. (2024), and our study supported their results.

CONCLUSION

Overall, *Ruellia prostrata* leaves display a high concentration of phytochemicals, which may have an antioxidant effect on several in vitro models. The concentration of the leaf extract was found to have a significant impact on the antioxidant activity. The presence of bioactive substances such phenols, terpenoids, alkaloids, and flavonoids can account for such action. Using advanced analytical methods like HPLC, GC-MS, and antioxidant analysis, the current study offers solid scientific data on the phytochemical characteristics of *Ruellia prostrata* leaves. In-vivo research, the isolation and

characterisation of specific bioactive chemicals, and comprehensive toxicity study are the anticipated future research topics. Modern technologies and computation models are aiding the incorporation of more efficient, ethical and human-relevant methods into these developments, which are becoming more informed.

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