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Comparative antioxidant, antibacterial and phytochemical analysis of roots, stems, leaves and seeds from *Cleome rutidosperma* DC

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ABSTRACT: The emerging microbial infections and their resistance to the existing antibiotics lead to discovering novel compounds, primarily from medicinal plants with secondary metabolites having several bioactive potentials, including antioxidants. The current investigation aims to measure the antioxidant and antibacterial efficacy of ethanolic extracts from roots, stems, leaves and seeds of *Cleome rutidosperma*. The extracts were subjected to quantitative (total phenolic and flavonoid), qualitative phytochemical studies, and functional groups identification by FT-IR analysis. The extract of leaves showed the highest total antioxidant (54.21±1.56 mg ABAE/g), DPPH (62.92 ±1.94 mg GAEs/g), and FRAP (71.64 ±2.02 mg GAEs/g) activity among the all-tested parts. The antibacterial efficacy of extracts was determined by the microdilution bioassay method, which demonstrated that G(+ve) bacteria appear to be more susceptible to the crude extracts than G (-ve) bacteria. The qualitative phytochemical screening-detected alkaloids, flavonoids, phenols, sugars, proteins, saponins, sterols, tannins, and terpenoids. The leaves have the highest levels of phenolics (70.451.23 mg GAE/g DW) and flavonoids (32.261.12 mg RE/g DW) among the all-tested parts. The extracts' functional group was validated using the FT-IR spectra. Polyphenols, flavonoids, and tannins were identified in the crude extracts. These findings imply that *C. rutidoserma* could be a promising candidate for further research into infectious illness treatment and as a resource of novel antioxidants in nutraceutical and biopharmaceutical industries as a functional additive.

1. INTRODUCTION

For the past few decades, researchers have been examining the biological properties of medicinal and food plants in the hopes of discovering a cure for a variety of modern diseases and a way to delay the onset of ageing symptoms. Free radicals and other reactive oxygens are highly reactive atoms or groups of atoms that contain an unpaired electron. The term "reactive oxygen species" (ROS) refers to oxygen derivatives that have the potential to be reactive (e.g., $O_2^{-}\cdot$, H_2O_2,·OH, and $^{\bullet}NO$). ROS are involved in oxidative damage to essential cellular components like proteins, lipids, lipoproteins, and DNA, which frequently occurs in biological systems and is associated with the etiology of diabetes, atherosclerosis, arthritis, cancer, cardiac problems, and neurological diseases, as well as the aging process (Farber, 1994; Halliwell & Gutteridge, 1985; Venkatesh & Dorai, 2011). Antioxidants can assist in preventing the oxidation of essential cellular components and guard against free radical damage. The scientific community is looking for new antioxidant agents with less deleterious effects than existing synthetic antioxidants (butylated hydroxyanisole and butylated hydroxytoluene) (Bajpai et al., 2014; Jun et al., 2014). Another primary public health concern is antibiotic resistance, particularly in developing nations where transmittable ailments are the primary reason for death (N.D. Silva et al., 2016).

Numerous plants have been utilized as alternative medicines for many infections, ailments, and food preservatives since ancient times, implying the existence of antimicrobial and antioxidant elements (Panovska et al., 2005). Plant-derived antioxidants guard against oxidative stress by discarding ROS and RNS (reactive nitrogen species). They also suppress the polymerization chain reaction that free radicals initiate (Ahmed et al., 2015; Bianco & Uccella, 2000; Céspedes et al., 2008; Lafka et al., 2007; O.A. Silva et al., 2012). Nearly 200 species

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are found in the genus *Cleome*, the Capparidaceae family. Since ancient times *Cleome* species has been used in folk medicine for its ethnomedicinal properties, including antimicrobial, antidiabetic, anthelmintic, anti-inflammatory, anticonvulsant, antipyretic, antidiarrheal, and carminative properties, as well as in the therapy of wounds, epilepsy, convulsions, spasm, pain, and skin ailments (Archi et al., 2016; U. Bose et al., 2011; Devi et al., 2002; Motaal et al., 2011; Parimaladevi et al., 2003; Thomas et al., 2014). So far, there is no literature on the comparative antioxidant and antimicrobial potential of different parts of *C. rutidosperma*. Therefore this study aimed to determine the antioxidant and antibacterial potential of ethanolic extracts from roots, stems, leaves and seeds of *C.rutidosperma* and to reveal the functional groups present in the test plant using FTIR, which possibly open a new perspective for rising novel products such as medicine.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Sigma-Aldrich provided the DPPH. All other compounds were of analytical quality and were obtained from Qualigens – Thermo Fisher Scientific, Mumbai, India.

2.2. Plant material

Cleome rutidosperma DC was collected in January 2020 on the campus of A.V.C. College in Mannampandal, Mayiladuthurai, Tamil Nadu, India. The root, stem, leaves, and seeds of the plant were removed. After thorough rinsing, the samples were dried in the shade until they attained a consistent weight. Using a mixer grinder, the dry samples were crushed to a fine powder and stored for later use. The extract preparation and analytical methods followed in this study were given in the supplementary file.

2.3. Statistical analysis

All tests were performed in triplicate, and the findings are expressed as mean ± SD. The data were evaluated statistically using one-way ANOVA with Tukey's ($p < 0.01$) test using the SPSS v22.

3. RESULTS AND DISCUSSION

3.1. Total antioxidant activity

Table 1 shows the findings of various assays (total antioxidant, DPPH, and FRAP) used to investigate the antioxidant potential of ethanolic extracts from *C. rutidosperma*. The total antioxidant activity of different parts of *C.rutidosperma* extracts ranged from 30.63 ± 1.34 to 54.21 ± 1.56 mg ABAE/g, with higher antioxidant activity in leaves and lower antioxidant activity in roots. In terms of total antioxidant activity, there was a significant ($p < 0.01$) difference across the different parts of *C. rutidosperma*. Molybdenum VI is reduced in the phosphomolybdenum assay to generate a green phosphate/Mo^{5+} combination.

3.2. DPPH radical scavenging activity

The radical scavenging potential of the plant components evaluated here showed significant ($p < 0.01$) differences. DPPH radical scavenging activity ranged from 36.62 ± 1.62 to 62.92 ± 1.94 mg GAEs/g. The leaves had a significantly higher radical scavenging potential than the roots, significantly lower. The capacity of antioxidants to donate hydrogen is thought to be the mechanism by which they scavenge DPPH radicals. DPPH utilises an electron or hydrogen radical to create a stable diamagnetic molecule. When DPPH is combined with a hydrogen atom donor, a stable non-radical form of DPPH is generated, and a colour shifts from violet to light yellow. (Molyneux, 2004). As a result, DPPH is commonly used as a free radical in evaluating reducing chemicals (Cotelle et al., 1996) and is a useful reagent for evaluating compounds' free radical scavenging characteristics (Duan et al., 2006). According to Ghimire et al. (2011), the existence of bioactive chemicals such as phenolics, flavonoids, and tannins in different plant sections could reflect the varying scavenging activities. The perusal of available literature indicates a clear link between the radical scavenging ability of plant extracts and phytochemical contents (Arumugam et al., 2019, 2020; Namvar et al., 2017). The current findings also support the view of Yingming et al. (2004), who found that extracts with a high phenolic content were more effective in scavenging free radicals. Nonetheless, other extracts with lower phenolic levels demonstrated considerable activity, suggesting that other secondary metabolites may contribute to the scavenging activity.

3.3. Ferrous reducing ability (FRAP)

The ferrous reducing ability of different parts of *C. rutidosperma*, measured in Gallic acid equivalent (mg/g), was shown to be significantly different ($p < 0.01$) and increased in the order of roots, stems, seeds, and leaves in the current investigation. Like other antioxidant activities, the leaves (71.64 ± 2.02 mg GAEs/g) demonstrated a stronger ability to reduce Fe3+ than roots (42.51 ± 1.08 mg GAEs/g). In the FRAP assay, antioxidants in the samples used a redox-linked colorimetric mechanism involving single electron transfer to convert ferric (III) to ferrous (II) (Li et al., 2006). Flavonoids have a perfect structure for scavenging free radicals because they include several hydroxyls that act as hydrogen donors, making them potent antioxidant agents (Marya et al., 2011). According to Barros et al. (2007), the existence of reductones, which act as antioxidants by donating a hydrogen atom to the free radical chain, is associated with the presence of reducing capabilities.

3.4. Antibacterial activity

Table 2 summarises the antibacterial efficacy of ethanol extracts from *C.rutidosperma* roots, stems, leaves, and seeds against six human pathogens. The MIC was estimated over the test pathogens. The lowest MIC values among the four extracts indicated the highest antibacterial activity. All *C. rutidosperma* extracts exhibited a broad antibacterial activity, as they inhibited all microorganisms tested. The range of MIC

Table 1
Antioxidant activity of ethanolic extracts from *C.rutidosperma.*

Assays	Roots	Stems	Leaves	Seeds
Total antioxidant activity (mg ABAE/g)^{x}	30.63±1.34^{d}	43.22±1.85^{c}	54.21±1.56^{a}	47.22± 1.24^{b}
DPPH radical (mg GAEs/g)^{y}	36.62±1.62^{c}	49.12±1.33^{b}	62.92±1.94^{a}	51.51±1.55^{b}
FRAP reducing power (mg GAEs/g)^{y}	42.51±1.08^{c}	54.85±2.45^{b}	71.64±2.02^{a}	58.12± 1.43^{b}
Total phenolic (mg GAEs/g)^{y}	37.23±0.62^{c}	49.31±1.44^{b}	70.45 ±1.23^{a}	51.22±1.31^{b}
Total flavonoid (mg REs/g)^{z}	20.11±0.89^{c}	25.33±1.62^{b}	32.26±1.12^{a}	27.41±1.52^{b}

Different subscripts in the same column indicate significant difference ($p < 0.01$).

to inhibit *Salmonella typhii* (1.56 - 6.25 μg/mL), *Escherichia coli* (1.56 - 6.25 μg/mL), *Pseudomonas aeruginosa* (3.125 - 6.25 μg/mL), *Staphylococus aureus* (6.25 - 3.125 μg/mL), *Streptococcus pyogens* (1.56 - 3.125 μg/mL) and *Bacillus subtilis* (0.78 - 3.125 μg/mL). Significant inhibition was defined as MIC less than or equal to 1.56 mg/mL. The extracts were more sensitive to G(+) bacteria than G(-) bacteria. *B. subtilis* and *S. pyogens* were most susceptible to the extracts, and the rest of the pathogens showed almost similar kinds of MIC values. Similarly, N.D. Silva et al. (2016) found that *Cleome spinosa* extracts were more active against G(+) bacteria. McNeil et al. (2018) reported that essential oil from above-ground portions of *C. rutidosperma* displayed antibacterial and antifungal action, with the most substantial inhibitory effect against *Bacillus cereus*. The ethanolic extract of *C. rutidosperma* leaves has higher antimicrobial activity than the aqueous extract (Ghosh et al., 2019).

Table 2
Minimum inhibitory concentrations (MIC - μg/mL) of ethanolic extracts from *C. rutidosperma* against selected bacterial pathogens.

Pathogens	Roots	Stem	Leaves	Seeds	Gentamycin
Salmonella typhii	6.25	12.5	3.12	6.25	0.195
Escherichia coli	12.5	12.5	6.25	6.25	0.195
Pseudomonas aeruginosa	6.25	6.25	3.12	3.12	0.195
Bacillus subtilis	1.56	1.56	0.39	0.78	0.049
Staphylococcus aureus	1.56	1.56	0.39	0.78	0.024
Streptococcus pyogens	3.12	3.12	1.56	3.12	0.097

In the present study, *Bacillus* sp. was most susceptible to the extracts. Against all the tested bacterial pathogens, leaves extract of *C. rutidosperma* had the highest inhibition effects. When compared to the antibacterial activity of standard antibiotics (gentamicin), the antimicrobial potential of the extracts was in the following order: roots > stems > seeds >leaves. Numerous secondary metabolites such as alkaloids, flavonoids, phenols, saponins, steroids, and others may be responsible for medicinal plants' therapeutic powers. Many fungi, bacteria, and viruses are inhibited by polyphenolic chemicals found in medicinal plants (Sodipo et al., 2000). A. Bose et al. (2007) reported that an ethanolic extract of *C. rutidosperma* revealed the presence of lipids, steroids, terpenoids, flavonoids, tannins, saponins and sugars. According to Donkor et al. (2014), alkaloids and tannins presented in *C. viscosa* are associated with antibacterial activity. The presence of tannins, triterpenoids, and flavonoids in ethanol extract may explain its antibacterial properties. Tannins have been demonstrated to form irreversible compounds with prolene-rich proteins, resulting in the inhibition of cell wall synthesis (Chandrika & Chellaram, 2015; Mamtha et al., 2004).

3.5. Qualitative phytochemical analysis

Analysis of the extract yield showed that the *C. rutidosperma* leaves had the highest percentage compared to roots, stems and seeds (**Table S1**, Appendix A). A total of 13 qualitative phytochemical tests were carried out to detect their presence in plant extracts. The extracts contained alkaloids, flavonoids, phenols, sugars, proteins, saponins, sterols, tannins, and terpenoids, which conferred to the qualitative phytochemical screening study. Furthermore, glycosides were found in the stem, leaves, and roots, whereas coumarin was only found in the roots. On the other hand, quinones were found in none of the extracts (**Table S1**, Appendix A). Secondary metabolites found in *C. rutidosperma* samples include saponins, flavonoids, tannins, coumarins, and terpenoids, all of which have antibacterial and antifungal properties (Hayek et al., 2013).

3.6. Total phenolic and flavonoid contents

The therapeutic qualities of medicinal plants are due to secondary metabolites produced by the plants. Phenolic compounds (flavonoids, phenolic acids, quinones, phenylpropanoids, tannins, lignins, and hydroxyl compounds) are among the most diverse families of secondary metabolites found in natural products (Alu'datt et al., 2017; Nile et al., 2017). Table 1 shows the total phenolic and flavonoid content of different parts of ethanolic extracts from *C. rutidosperma*. The total phenolic content ranged between 37.230.62mg GAE/g DW and 70.451.23 mg GAE/g DW. Total phenolic content was significantly higher ($p < 0.01$) in leaves than in other parts tested. Phenolic chemicals are a diverse category of secondary metabolites that vary in distribution and concentration across and within plant species (Robards et al., 1999). Like phenol, the leaves had a significantly ($p < 0.01$) greater total flavonoid concentration. The total flavonoid content ranged between 20.110.89mg RE/g DW and 32.261.12mg RE/g DW. Ghosh et al. (2019) reported that the total phenolic (121.671.82 mg GAE/g DW) and flavonoids (65.080.98 mg QE/g DW) content

of ethanolic extracts of the same species was more significant than the concentration found in this study. According to D. Silva et al. (2006), a higher concentration of phenolics and flavonoids in plant leaves might result from photosynthesis. Although no significant ($p < 0.01$) variation was observed in total phenolic and flavonoid contents between the stem and seed extracts of *C.rutidosperma*. Polyphenolic compounds, such as total phenolic and total flavonoids, have been linked to many biological functions, including antioxidants (Céspedes et al., 2010; Djeridane et al., 2006; Lamien-Meda et al., 2008; Meda et al., 2013), antimicrobial activities (Shan et al., 2007). The capacity of phenolic compounds and flavonoids to scavenge hazardous free radicals and reactive species is well documented (Hall & Cuppett, 1997; Jørgensen et al., 1999; Pietta, 2000; Rice-Evans et al., 1997). Flavonoids play a significant role in both human and animal nutrition. They are a well-known class of natural chemicals that have been shown in various studies to have antioxidant and hepatoprotective properties (Bratkov et al., 2016; Krasteva et al., 2016; Pistelli, 2002). Plant secondary metabolites involve a range of biological actions, and therefore their regular consumption can have beneficial and harmful health repercussions (Stobiecki & Kachlicki, 2006).

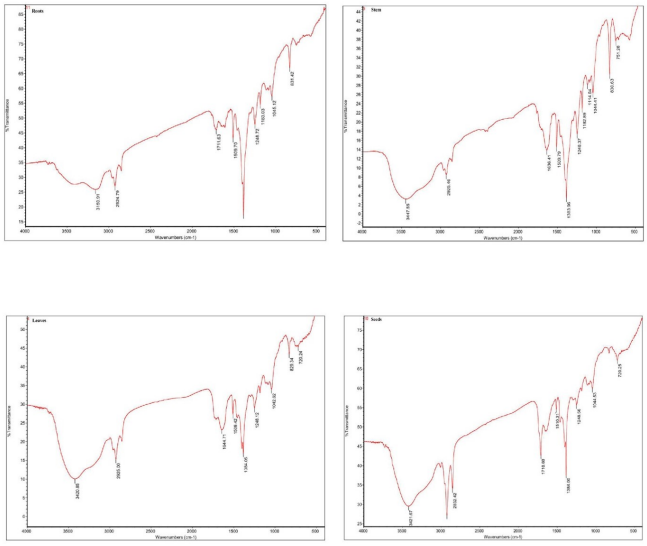

Figure 1. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of ethanolic extracts from *C. rutidosperma*

3.7. Fourier Transform Infrared Spectroscopy analysis

Figure 1 and **Table S2**, Appendix A exhibit sample IR spectra analyses in the mid-infrared region (4000-400 cm^{-1}) for aqueous ethanolic extracts from *C. rutidoseprma*. For all plant parts examined, similar peaks were found at 2921, 1509, 1383, 1248, and 1044 cm^{-1}. Absorbance bands at 3420 - 3447 cm^{-1} were identified as polyphenols in the plant parts used in this study. The O-H stretching vibrations between 3447 and 3159 cm^{-1} confirmed the presence of water, alcohol, and phenols in the sample (Larkin, 2011). The C=O stretching vibration in carbonyl compounds, defined by the presence of flavonoids in the samples, is ascribed to the absorption peak at 1644 – 1636 cm^{-1} (Socrates, 2001). The absorption bands at 2921 cm^{-1} and 2925 cm^{-1} correspond to CH_2 group C-H stretching, indicating the presence of several amino acids. The presence of aliphatic and aromatic CH groups in these compounds may also be indicated by these bands (Ramamurthy & Kannan, 2007; Sivakesava & Irudayaraj, 2000). The bands at 2925 and 2921 cm^{-1} are caused by asymmetric stretching of the aromatic rings due to methyl (-CH_3) and methylene (-CH_2-) substitutions, respectively (Socrates, 2001). Absorption bands at 1509 cm^{-1} suggest the existence of the benzene ring in aromatic chemicals in all plant parts of *C. rutidosperma*. The absorption band at 1044 cm^{-1} in this study could be the stretching vibration of the C-O-C group of esters Vlachos et al. (2006).

4. CONCLUSION

The plants under investigation can provide adequate protection against oxidative stress through complementary systems such as free radical scavenging metal ion reduction and antibacterial activity. More research into phytochemical compound isolation through chromatographic techniques demonstrating antioxidant, antimicrobial, and other pharmacological activity is required before being used as valuable additives in the nutraceutical and biopharmaceutical sectors.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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A. APPENDIX A. SUPPLEMENTARY INFORMATION

Supplementary information to this article can be found online at https://doi.org/10.53365/nrfhh/146009.

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