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Phytochemical Screening, Anti-oxidant and Anti-microbial Activity of leaves of *Cleome rutidosperma* DC. (Cleomaceae)

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Abstract

Cleome rutidosperma DC. (Nil Hurhure), belonging to the family Cleomaceae being used for the treatment of oxidative stress related and microbial infected diseases. The research study was carried out to estimate the phytochemical components and to investigate anti-oxidant and anti-microbial activity of aqueous and 70% ethanolic extracts of leaves of the plant. The anti-oxidant and anti-microbial activities of the plant can be depended mainly on bioactive compounds. So their quantitative and qualitative experimentation was undertaken. The anti-microbial assay was done by disc diffusion method. The total phenolic content of aqueous and 70% ethanolic extracts showed the values 102.70±2.73, and 121.67±1.82 mg Gallic acid equivalent/g dry weight and the total flavonoids content of aqueous and 70% ethanolic extract showed the values 36.93±1.16 and 65.08±0.98 mg Quercetin equivalent/g dry weight, and the total tannin content for aqueous and 70% ethanolic extract 19.05±0.48 and 20.03±0.30 mg Tannic acid equivalent/g dry weight, respectively. Research studies showed that 70% ethanolic extract has higher anti-oxidant and anti-microbial activities. The results revealed that leaves possess the bioactive substances which may be responsible for the treatment of oxidative stress related and microbial infected diseases. Among these results, 70% ethanolic extract has more potent than an aqueous decoction.

Keywords: *Cleome rutidosperma*, Polyphenols, Flavonoids, Anti-oxidants, Anti-microbial.

INTRODUCTION

Traditional medicines were used for the treatment of animals from ancient time. It is cost effective as well as has better adjustment with the human biological system and has minimal side effects. The demand for ethnomedicinal treatment is increasing. Phytochemical screening is essential to find out the new sources of natural drugs for therapeutic and industrial purposes. The present course of study is highlighting on a medicinal herbaceous weed that has a vast use in the traditional treatment system. The plant is very less characterized and reported with anti-oxidant and anti-microbial activity [1, 2].

***Cleome rutidosperma* DC.** is an annual herbaceous plant, and it is commonly known as **Fringed Spider Flower,** and in Bengali, it is known as **Nil or Beguni Hurhure**. It is a low growing herb up to 100 cm tall and found in waste grounds or humid places with trifoliate leaves. The plant is native to Tropical Africa. The leaves are eaten as a vegetable or added to soup and have a bitter taste such as mustard. Leaf sap is applied to cure earache, irritated skin, prickly heat, and convulsions. Extensive literature study suggested that this plant has different biological activities like anti-bacterial, wound healing, anti-oxidant, anti-diabetic, diuretic and laxative activity, etc. [1].

Medicinal properties of *Cleome rutidosperma* are associated with many bioactive compounds. The plant has a significant property to scavenge reactive oxygen species (ROS). This ROS is the ultimate source of oxidative stress-related diseases. Phytocompounds are the naturally bioactive molecules which are distributed in plants and that have been reported to exhibit various biological activities. Therefore, it is necessary to detect and estimate those bioactive compounds [1, 3, 4].

In the present study anti-microbial activity of this medicinal herb was done by using disc diffusion assay. Phytochemical screening and anti-oxidant activity determination were also carried out. In the research study, we have used water and 70% ethanol as solvents to prepare the extracts from the leaves of the plant.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals and reagents used in the experiments were of analytical grade. Folin-ciocalteu reagent, aluminum chloride, and ascorbic acid were obtained from Merck Life Science Private Limited, Mumbai. Gallic acid and hydrogen peroxide were purchased from SD Fine-Chem Limited, Mumbai. Quercetin, DPPH, tannic acid was obtained from Sisco Research Laboratories Pvt. Ltd., Maharashtra, and ABTS from Tokyo Chemical Industry Co. Ltd., Japan.

Collection and Extraction

Green leaf of the plant was collected from Salt Lake City, Kolkata, and West Bengal, India and authenticated by Botanical Survey of India, Central National Herbarium, Shibpur, Howrah, and West Bengal, India. Leaves of the plant were washed with distilled water and dried at room temperature for 25 days under shade. Leaves made powdered and extracted using mortar and pestle by two different solvents water and 70% ethanol, 50 ml each for 1 g of powder. The solution of extract was stored at 4°C and diluted for further studies according to the needs for the specific assay.

Phytochemical Screening

Test for Alkaloids

To detect alkaloids standard method was used with slight modifications. The reaction was considered positive when maroon or reddish brown precipitation was formed [5, 6].

Test for Flavonoids
To detect flavanoids standard method was used with slight modifications. The intense yellow color was formed which becomes colorless on the addition of dilute HCl, and it indicates the presence of flavonoids [7].

Test for Reducing Sugars
To detect reducing sugars standard protocol was used and brick red precipitate indicates the presence of reducing sugars [5, 8].

Test for Phenols
To detect phenolic compounds standard protocol was used. Formation of dark-greenish, blue to dark-blue or even blackish color indicating the presence of phenols [9].

Test for Carbohydrates
To detect carbohydrate standard method was used with slight modifications. Formation of a red or dull violet or purple color at the junction of the two layers was observed, and it signifies the presence of carbohydrates [10].

Test for Sterols
To detect phytosterols standard method was used. Formation of dark pink or red color signifies the presence of steroids [11].

Test for Terpenoids
To detect terpenoids standard protocol was used. A reddish brown precipitate produced indicated the presence of terpenoids [10].

Test for Amino acids
To detect amino acids standard protocol was used with slight modifications. Formation of purple color indicated the presence of amino acids [12].

Test for Proteins
To detect protein standard method was used with slight modifications. Formation of purplish violet color indicates the presence of protein [13].

Test for Tannins
To detect tannins standard protocol was used with slight modifications. In the reaction mixture, a blue color formed for Gallic tannins and green-black color indicates the presence for Catecholic tannins [8].

Phytochemical Analysis

Quantification of Total Phenolic Compounds
The total polyphenolic contents were determined according to the Folin-Ciocalteu method with slight modification. Gallic acid was used as a standard curve. The absorbance read at 765 nm. The content of polyphenolic compounds of the samples was expressed as mg Gallic Acid Equivalent/g dry weight [14].

Quantification of Total Flavonoids Content
Total flavonoid content was studied by the aluminium chloride colorimetric assay with slight modification. The absorbance was read at 510 nm. Quercetin was used as standard. The total flavonoid content was expressed as mg Quercetin Equivalent/g dry weight [15].

Quantification of Total Tannins
The total tannin content was measured by using standard protocol. Tannic acid used as a standard reagent. The absorbance was taken at 500 nm. Total tannin content was expressed in terms of mg Tannic Acid Equivalent/g dry weight [16].

Determination of Anti-oxidant Property

DPPH Radical Scavenging Test
The free radical scavenging activity was evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by the standard protocol with slight modifications. The standard curve was made using Ascorbic acid. Absorbance was read at 517 nm. DPPH radical scavenging activity was expressed in terms of Ascorbic Acid Equivalent, as a percentage of inhibition calculated by the following formula: [17]

% Inhibition of DPPH= Abs of control – Abs of sample/ Abs of control*100

ABTS Radical Scavenging Assay
ABTS radical cation decolorization assay was used to study the free radical scavenging activity of plant extracts by using standard protocol with slight modifications. Absorbance was taken at 734 nm. Ascorbic acid was used as a standard reagent. ABTS radical scavenging activity was expressed in terms Ascorbic Acid Equivalent, as percentage inhibition calculated by the formula: [18]

% Inhibition of ABTS= Abs of control – Abs of sample/ Abs of control*100

H_2O_2 Radical Scavenging Assay
Hydrogen peroxide scavenging ability was measured by using a standard method with slight modification. The absorbance was taken at 230 nm. Gallic acid was used as a standard reagent. H_2O_2 radical scavenging activity was expressed in terms Gallic Acid Equivalent, as percentage inhibition calculated by the formula: [19]

% Inhibition of H_2O_2= Abs of control – Abs of sample/ Abs of control*100

Determination of Anti-microbial Activity
The anti-microbial activity was carried out by using disc diffusion assay on nutrient agar plates with the paper disc was plotted into them. Culture obtained was then spread across the agar and allowed to stand for 10-15 min, under sterile condition. 20µl of the sample extract was given into the disc with sterile distilled water and 70% ethanol as control. Then, the plates were incubated 37°C for 18 hrs. Zone of inhibition was measured. One gram-positive and two gram-negative organisms namely, *Staphylococcus aureus,* and *Escherichia coli, Vibrio cholerae,* respectively were tested for anti-microbial activity against the control double distilled water and 70% ethanol [20].

Statistical Analysis
All the experimental measurements (except anti-microbial activity assay) were performed in triplicate and expressed as the average ± standard deviations. The magnitude of the means, standard curve, standard errors, and standard deviations were calculated by using MS Excel Software.

RESULTS AND DISCUSSION

Qualitative Assay
Results obtained from qualitative screening from leaf aqueous and 70% ethanol extracts of *Cleome rutidosperma*, is presented in Table 1. A total of 10 qualitative tests were done to detect different phytochemicals which are present in this medicinal herb. Among them, four were present in both the extracts. These were flavonoids, phenols, proteins, and tannins. 70% of ethanol extracts showed the highest

presence of compounds. Eight phytochemicals were present out of 10 tests. The results indicate that the experimental plant holds huge prospects as a source of therapeutically and pharmaceutically critical bioactive molecules. Therefore, Estimation of these phytomolecules becomes vital point [4].

TABLE 1: RESULTS OF PHYTOCHEMICAL SCREENING

Test Name	Solvent Name	
	W	E
Alkaloids	-	+
Flavonoids	+	+
Reducing sugars	-	+
Polyphenols	+	+
Carbohydrate	-	+
Steroids	-	+
Triterpene	+	-
Amino Acid	+	-
Protein	+	+
Tannin	+	+

WHERE, "+"PRESENT, "-" ABSENT

Quantitative Assay

The total polyphenolic content for *Cleome rutidosperma* was quantified to be 102.70±2.73 mg GAE/g dry weight for aqueous extract and 121.67±1.82 mg GAE/g dry weights for 70% ethanol extract (Figure 1). Polyphenolic compounds are reactive species towards oxidation and regulate the physiological activity. The oxidation process and free radicals generation lead to microbial infections, diabetes, cancer, and other diseases. The activity of polyphenols against those oxidative stress-related causing processes can have therapeutic application in the pharmaceutical industry. Plants having more phenolic content show significant anti-oxidant properties [14, 17, 18, 19, 20, 21].

The total flavonoids content was quantified to be 36.93±1.16 mg QE/g dry weight for aqueous extracts and 65.08±0.98 mg QE/g dry weight for 70% ethanolic extracts (Figure 2). The anti-oxidative activities of flavonoids are due to various mechanisms, like scavenging of free radicals, chelation of metal ions (e.g., iron and copper), and inhibition of enzymes which are responsible for a free radical generation. Depending on their specific structure, flavonoids compounds can inhibit all the possible reactive oxygen species [15, 17, 18, 19, 20, 21].

The total tannin content was quantified to be 19.05±0.48 mg TAE/g dry weight for aqueous extracts and 20.03±0.30 mg TAE/g dry weight for 70% ethanol extracts (Figure 3). Tannins are mainly found in the stem of many plants rather than leaves. The high content of tannins reflects the presence of the critical anti-oxidant and anti-microbial properties. The tannin-protein complex shows the persistent anti-oxidant and anti-microbial property [16, 17, 18, 19, 20, 21].

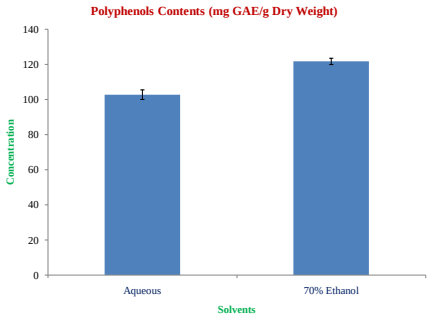

FIGURE 1: POLYPHENOL CONTENT (mg GAE/g DRY WEIGHT)

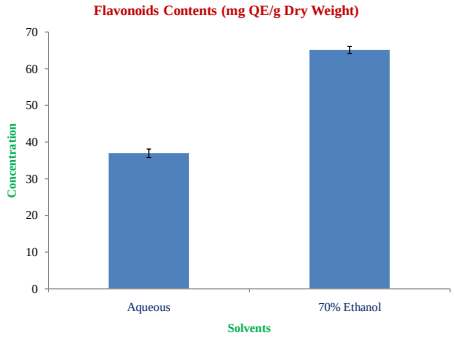

FIGURE 2: FLAVONOIDS CONTENT (mg QE/g DRY WEIGHT)

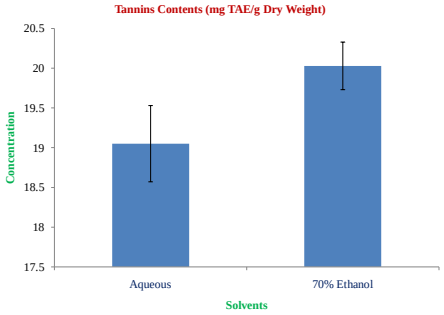

FIGURE 3: TANNIN CONTENT (mg TAE/g DRY WEIGHT)

Inhibition and Concentration Values for Radical Scavenging Assays

Inhibition concentration is the number of free radicals scavenged in the evaluation of the antioxidant property. Phytomolecules act as anti-oxidants by scavenging the free radicals. DPPH is a free radical and is widely used to investigate the free radical scavenging capacity of anti-oxidants. DPPH assay is based on the reduction of stable free radicals in methanol solution in the presence of hydrogen–donating anti-oxidants due to the formation of the non-radical form of standard stable free radical [15, 17]. The inhibition percentage of DPPH radical scavenging assay was found to be 71.29±0.27% for aqueous extract and 74.53±0.25% for 70% ethanol extract (Figure 4); as compared to inhibition percentage for standard reagent. 70% ethanol extract shows higher inhibition concentration and scavenged maximum amount of radicals as compared to aqueous leaf extract (Figure 5).

The scavenging capacity of the ABTS cation radical has been compared with ascorbic acid. Potassium persulfate was used to give a stable form of ABTS radical cation in the assay [18]. The inhibition percentage of ABTS radical scavenging assay was found to be 57.62±0.46% for aqueous extract and 63.91±0.64% for 70% ethanol extract (Figure 6); as compared to inhibition percentage for standard reagent. 70% ethanol extract shows higher inhibition concentration and scavenged maximum amount of radicals as compared to aqueous leaf extract (Figure 7).

H_2O_2 assay is based on the reduction of stable free radicals in phosphate buffer solution (pH 6.8) in the presence of hydrogen-donating anti-oxidants due to the formation of the non-radical form of standard stable free radical [19]. The inhibition percentage of H_2O_2 radical scavenging assay was found to be 70.10±0.37% for aqueous extract and 71.29±0.16% for 70% ethanol extract (Figure 8); as compared to inhibition percentage for standard reagent. 70% ethanol extract shows higher inhibition concentration and scavenged maximum amount of radicals as compared to aqueous leaf extract (Figure 9). These *in vitro* anti-oxidants research study findings support the previous investigations as well which concluded that the anti-oxidant properties are mainly dependent on several bioactive compounds such as polyphenols, flavonoids and tannins [21, 22, 23, 24].

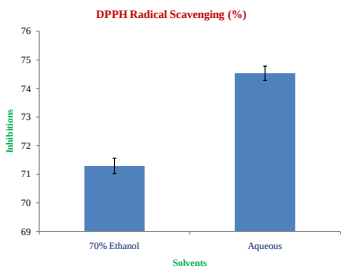

FIGURE 4: DPPH RADICAL SCAVENVING (%)

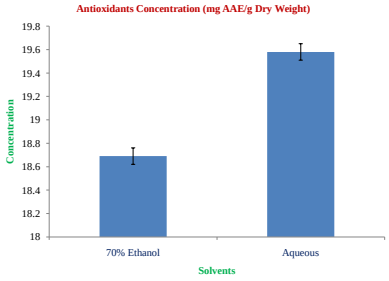

FIGURE 5: DPPH INHIBITION CONCENTRATION (mg AAE/g DRY WEIGHT)

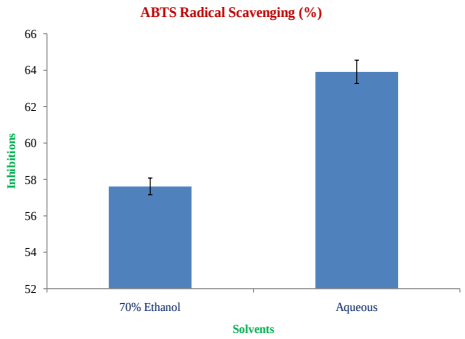

FIGURE 6: ABTS RADICAL SCAVENGING (%)

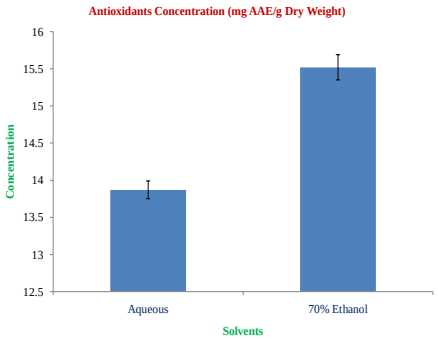

FIGURE 7: ABTS INHIBITION CONCENTRATION (mg AAE/g DRY WEIGHT)

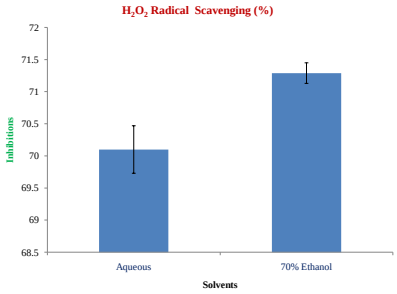

FIGURE 8: H_2O_2 RADICAL SCAVENGING (%)

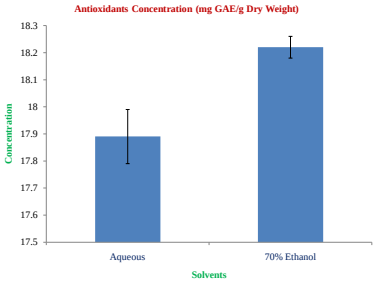

FIGURE 9: H_2O_2 INHIBITION CONCENTRATION (mg GAE/g DRY WEIGHT)

Anti-microbial Evaluation

Zone of inhibition shown by aqueous leaf extract of *Cleome rutidosperma* DC. Indicates the presence of no anti-microbial activity. 70% ethanol leaves extract of the plant showed better zone of inhibition compared to aqueous decoctions. These indicated that plant extracts had a significant anti-bacterial property in 70% ethanol than traditionally claimed aqueous decoctions. From the present study, it is concluded that these zones of inhibition may be due to the presence of higher bioactive contents such as flavonoids and tannins which is highly related with the anti-microbial activity of the plant extracts which supports the previous study as well. Bioactive compounds mainly act as the anti-microbial agent (Figure 10: A, B, C, D, E and Table 2) [4, 24, 25].

Table 2: Anti-microbial activity of *Cleome rutidosperma* DC. : Zone of Inhibitions (mm)

Organisms			Zone of Inhibition (mm)			
	Distilled water (control)	Aqueous extract	Net Zone	70% Ethanol (control)	70% Ethanol extract	Net Zone
Escherichia coli	0	Nil	Nil	0	11.70	11.70
Staphylococcus aureus	0	Nil	Nil	6	9.70	3.70
Vibrio cholerae	0	Nil	Nil	0	9.0	9.0

Anti-microbial Activity : Zone of Inhibition (mm)

Figure A: EC 70% EtOH

Figure B: SA 70% EtOH

Figure C: VC 70% EtOH

Figure D: EC Aqueous

Figure E: SA Aqueous

FIGURE 10: ANTI-MICROBIAL ACTIVITY: ZONE OF INHIBITION (MM)

CONCLUSION

In the current research study, the extracts of *Cleome rutidoperma* DC. were found to be rich in secondary plant metabolites and possess a significant amount of phytochemicals as well as it showed higher anti-oxidant and anti-microbial activity. The results showed the higher content of phytochemical constituents like polyphenols, flavonoids, tannins and higher anti-oxidant activity for DPPH, ABTS and H_2O_2 radical scavenging assays. The antioxidant activities of the leaf extracts may be attributed to their free radical-scavenging ability. Besides, polyphenolic substances and other phytochemicals appear to be responsible for the anti-oxidant properties of the extracts. The higher amount of flavonoid and tannin content is mainly responsible for significant anti-microbial activities, which supports previous research investigations as well. According to the results obtained from the present research investigation, the leaves are the vast source of natural phytochemicals, anti-oxidants and could be used to develop industrial or pharmaceutical products against various diseases such as microbial infections, inflammation, diabetes, cancer, hypertension, arthritis, and other oxidative stress-related diseases.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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