

Evaluation of Antioxidant, Cytotoxic, and Antibacterial Activities of *Dactyloctenium australe* Steud.

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Abstract

The widespread use of traditional medicine and medicinal plants as a normative basis for maintaining good health has been observed in the majority of developing countries. An *in vitro* antioxidant study of *Dactyloctenium australe* showed statistically significant ($p < 0.01$) antioxidant activity by scavenging free radicals, compared to the standard ascorbic acid. The IC₅₀ value for the chloroform soluble fraction (CHS) was 14.16 ± 0.11 $\mu\text{g/ml}$, followed by the carbon tetrachloride soluble fraction (CTS) with IC₅₀ values of 18.10 ± 1.09 $\mu\text{g/ml}$ and 24.54 ± 1.34 $\mu\text{g/ml}$. The CHS fraction also demonstrated the highest cytotoxic activity *in vitro*, with an LC₅₀ value of 15.14 $\mu\text{g/ml}$, compared to the standard vincristine sulfate (0.451 $\mu\text{g/ml}$). Furthermore, for antibacterial activity, the methanolic extract and its CHS fraction exhibited distinct zones of inhibition against Gram-positive bacteria, while the petroleum ether soluble fraction (PS) and CTS fraction also showed significant activity. *Dactyloctenium australe* exhibited notable antibacterial activity against *Staphylococcus aureus* (a Gram-positive bacterium) and *Salmonella paratyphi*, *Salmonella typhi* and *Vibrio cholerae* (Gram-negative bacteria), compared to the standard antibiotic ciprofloxacin.

Key words: Anti-oxidant, cytotoxic, anti-microbial, *Dactyloctenium australe*, ascorbic acid, vincristine sulfate and ciprofloxacin.

Introductions

It is widely believed that nature provides cures for many ailments. Plants and their extracts have been used since ancient times for their pharmacological properties. A wide variety of bioactive molecules that plants can produce are necessary for their vital physiological systems as well as their defense against pests including bugs, fungi, and herbivorous animals (Nweze *et al.*, 2004). Herbal medications are considered safe for many illnesses. Since ancient times, herbal remedies have served as the primary remedy in conventional medical systems. The traditions are still used today due to their biological advantages as well as their status in many cultures around the world and their significant contribution to preserving human health (Kirmani

et al., 2011). The widespread use of traditional medicine and medicinal plants as a normative basis for maintaining good health has been observed in the majority of developing countries. Therefore, identifying new potent and safe bioactive compounds is essential for drug discovery and development. Following a thorough evaluation of the literature, the plant *Dactyloctenium australe* (Steud.) was chosen for this study because it had received less attention in the past.

Materials and Methods

Plant material and extraction: *D. australe* samples were collected from Sylhet District, Bangladesh in, February, 2016 and was authenticated in Bangladesh National Herbarium, where a voucher

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specimen has been preserved representing this collection (Accession No. DACB-42771). Plant samples were washed thoroughly, sliced into small fragments, and dried at shed for one week. The dried samples were ground into a fine herbal powder. The powdered plant material was defatted using hexane extraction and subsequently dried at 37°C. The crude extract was partitioned using a modified Kupchan method, involving sequential extraction with petroleum ether, carbon tetrachloride, chloroform, and water, as described by Muhit et al. (2010).

Total phenolic content: Total phenolic content was determined using the Folin-Ciocalteu reagent method, with absorbance measured at 765 nm. The absorbance of the reaction mixture was recorded at 765 nm, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract, following the procedure of Skerget et al., (2005).

DPPH free radical scavenging assay: The antioxidant activity of the fractions was assessed using the DPPH free radical scavenging assay, following the method of Brand-Williams et al., (1995). The absorbance was measured at 517 nm, and the results were expressed as IC₅₀ values, representing the concentration required to scavenge 50% of the DPPH radicals.

Cytotoxic activity: Cytotoxic activity was evaluated using the brine shrimp lethality bioassay as described by Meyer et al., (1982). A series of concentrations was prepared for each fraction, and the mortality of brine shrimp larvae was recorded after 24 hours of exposure. LC₅₀ values were calculated using probit analysis to determine the concentration lethal to 50% of the larvae.

Antibacterial activity: The antibacterial activity of the fractions was assessed using the disc diffusion method, as described by Bauer and Tittle (1966). The bacterial strains tested included both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Salmonella typhi*) species. Discs impregnated with 30 µg of each fraction were placed on Mueller-Hinton agar plates inoculated with bacterial cultures, and zones of inhibition were measured after 24 hours of incubation at 37°C.

Ciprofloxacin (30 µg/disc) was used as the standard antibiotic.

Statistical analysis: All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation (SD).

Results and Discussion

The main objective of our current study was to determine the antioxidant activity, total antioxidant capacity, total phenolic content, and antimicrobial activity of four different fractions (Table-1).

Table 1. Amount of partitionates obtained from *D. australe* (25 g) methanolic extract.

Fraction	Weight (g)
PESF	3.9
CTCSF	2.7
CSF	3.6
AQSF	5.9

PESF=Petroleum ether soluble fraction, CTCSF= Carbon tetrachloride soluble fraction, CSF= Chloroform soluble fraction and AQSF= Aqueous soluble fraction.

These partitionates were then used to investigate the biological activities. The biological activities of these partitionates were then examined using the established protocol. These biological activities included antioxidant, brine shrimp lethality bioassay, antimicrobial, thrombolytic, membrane stabilizing, assessment of peripheral and central analgesic, hypoglycemic, anti-diarrheal, and CNS anti-depressant, among others.

According to literature survey there was no extensive biological research works on the *D. australe*. This study investigated the IC₅₀ values of five different fractions of the whole plant, highlighting the originality of this research. Therefore, Compounds with strong DPPH scavenging activity could serve as potential candidates for further pharmacological studies. In this connection the present research focused on DPPH free radical scavenging and total phenolic contents activities of this plant. In this study, the CHS fraction of *D. australe* exhibited statistically significant ($p <$

0.01) antioxidant activity by inhibiting free radical generation compared to ascorbic acid (AA) as the standard. The chloroform soluble fraction (CHS) of *Dactyloctenium australe* exhibited the highest antioxidant activity, with an IC_{50} value of 14.16 ± 0.11 $\mu\text{g/ml}$. This was followed by the carbon tetrachloride soluble fraction (CTS), with IC_{50} values of 18.10 ± 1.09 $\mu\text{g/ml}$ and 24.54 ± 1.34 $\mu\text{g/ml}$.

Comparatively, the standard ascorbic acid showed an IC_{50} value of 11.21 ± 1.43 $\mu\text{g/ml}$. On the other hand, in the plant sample, the amount of total phenolic content varies by extractive. The maximum phenolic concentration was reported in *D. australe* CHSF (52.56 ± 1.41 mg of GAE/g of extractives). For this experiment, all values were represented in the form of mean \pm SEM in the Table:2

Table 2. Antioxidant and cytotoxic activities of different extracts of *D. australe*.

Extract	Total phenolic content (mg GAE/g extract)	DPPH IC_{50} value ($\mu\text{g/ml}$)	LC_{50} value ($\mu\text{g/ml}$)
ME	31.09 ± 1.56	31.09 ± 1.56	29.20 ± 1.14
PSF	35.14 ± 1.23	35.14 ± 1.23	25.15 ± 1.04
CTSF	45.45 ± 1.43	45.45 ± 1.43	27.24 ± 1.17
CHSF	52.56 ± 1.41	14.16 ± 0.11	15.14 ± 1.07
ASF	29.67 ± 1.15	48.18 ± 1.34	51.34 ± 1.67
Standard	-	11.21 ± 1.43 (AA)	1.27 ± 0.87 (VS)

Today, antibiotic resistance poses a significant challenge for the global healthcare sector. Multidrug resistant microbes have drastically endangered the current antibacterial therapy. This necessity has dedicated us to explore a new source for antimicrobial drugs and therefore, plants could be a prominent candidate as they possess variety of bioactive compounds. By keeping pace with this, present study has been carried out to investigate the

antibacterial activity of five different fractionate of medicinal plant extracts against human pathogen. Antibacterial activity was investigated by means of disc diffusion technique and it was found that all the fractionates of the plant *D. australe* exhibited moderate activity against some Gm (+) ve and Gm (-) ve bacteria in comparison to ciprofloxacin as standard drug.

Table 3. Antibacterial activity (zone of inhibition) of different extracts of *D. australe*.

Bacteria	ME (mm)	PSF (mm)	CTSF (mm)	CHSF (mm)	ASF (mm)	Ciprofloxacin (mm)
Gram-positive bacteria						
<i>Staphylococcus aureus</i>	21.1	29.01	21.4	22.4	5.20	46.4
<i>Bacillus megaterium</i>	21.1	29.01	21.4	22.4	5.20	45.2
<i>Bacillus subtilis</i>	25.1	27.3	21.4	25.1	9.10	44.5
<i>Bacillus cereus</i>	22.6	25.1	21.5	21.3	4.80	46.4
Gram-negative bacteria						
<i>Escherichia coli</i>	22.7	31.9	20.1	24.1	3.40	45.5
<i>Pseudomonas aeruginosa</i>	22.1	34.7	22.2	25.1	5.30	44.7
<i>Salmonella typhi</i>	21.2	35.6	38.4	26.1	5.40	49.4
<i>Salmonella paratyphi</i>	28.3	23.2	24.2	23.7	7.20	45.4
<i>Vibrio cholerae</i>	22.1	33.40	31.5	22.8	6.50	46.4
<i>Shigella dysenteriae</i>	23.01	38.09	34.2	23.7	5.10	47.8

Using the disc diffusion technique, the antibacterial effectiveness against both Gm (+) ve and Gm (-) ve bacteria was evaluated. The results of this experiment showed that the methanolic bark extract and its PS and CTS fractionates of *D. australe* demonstrated substantial activity towards *S. aureus*, a Gm (+) ve bacteria, as well as *Staphylococcus aureus*, *Salmonella typhi*, and *V. cholera* Gm (-) ve bacteria whereas ciprofloxacin (30 µg/disc) used as a standard. The outcomes are listed in the Table:3.

Conclusion

This study demonstrates the antioxidant, cytotoxic, and antibacterial properties of *Dactyloctenium australe* Steud., supporting its traditional medicinal applications and providing a foundation for future pharmacological investigations into its bioactive compounds.

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