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POTENTIAL ROLE OF PLANT-BASED PROTEOLYTIC ENZYMES AND ANTIOXIDANTS IN CANCER PREVENTION: A COMPARATIVE ANALYSIS OF FRESH AND DRIED LEAF EXTRACTS OF *AMARANTHUS* SPECIES

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Cancer remains one of the leading global health burdens, with oxidative stress and chronic inflammation recognized as major contributors to its initiation and progression. Growing interest in plant-based therapeutics has highlighted the role of natural bioactive compounds—particularly proteolytic enzymes and antioxidants—in mitigating cancer risk through dietary means. In India, *Amaranthus* species are commonly consumed leafy vegetables known for their rich antioxidant content; however, their enzymatic and antioxidant contributions to cancer prevention remain insufficiently explored. This study aimed to evaluate and compare the antioxidant and enzymatic activities of fresh and dried *Amaranthus* leaves. Antioxidant capacity was assessed using the DPPH radical scavenging assay with both aqueous and methanolic extracts, while proteolytic enzyme activity was determined using a tyrosine-based assay. All experiments were performed in triplicate, and results are presented as mean ± standard deviation. Fresh leaves demonstrated higher antioxidant activity than dried leaves, with the greatest DPPH inhibition observed in the fresh methanolic extract (62.86 ± 1.25%), followed by the dried methanolic extract (56.92 ± 1.67%). Aqueous extracts showed comparatively lower activity overall. Similarly, proteolytic enzyme activity was greater in fresh leaves (33,600 ± 1,500 µmol/g/hr) than in dried leaves (28,200 ± 1,200 µmol/g/hr). These findings suggest that fresh *Amaranthus* leaves possess superior antioxidant and enzymatic properties. The combined presence of proteolytic enzymes and antioxidants underscores the potential of Amaranth leaves as a functional ingredient in cancer-preventive nutrition. The study provides valuable insights for developing plant-based dietary strategies and nutraceutical formulations aimed at reducing cancer risk through natural bioactive compounds.

Keywords: *Amaranthus* species, Antioxidant activity, Proteolytic enzymes, DPPH assay, Cancer prevention, Reactive oxygen species.

Introduction

Cancer is a complicated illness. Tumour initiation and cellular damage are largely caused by oxidative stress and persistent inflammation. Reactive oxygen species can increase the risk of cancer by causing DNA mutations and altering cellular signalling. Consuming foods high in antioxidants is becoming more well-known as a successful strategy for reducing oxidative stress and preventing cancer. Plants are a vast reservoir of bioactive compounds that exert diverse physiological and pharmacological effects. Phytochemicals, including polyphenols, flavonoids, alkaloids, terpenoids, and enzymes, are known for their ability to modulate biochemical pathways linked to inflammation, oxidative stress, and carcinogenesis.[1]

Amaranthus species, a nutrient-dense leafy vegetable and "positive millet," is a plant notable for its antioxidant and

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medicinal properties. Its leaves contain proteins, carotenoids, flavonoids (such as rutin), phenolic acids, and betalains, all associated with cytoprotective and anti-mutagenic activity.[2,3] *Amaranthus* extracts have demonstrated the ability to modulate oxidative stress and inhibit cancer cell proliferation.[4] They are inexpensive, readily available in the area, and rich in micronutrients such as calcium, iron, vitamins, and organic antioxidants. Compared to other healthful leafy greens, *Amaranthus* leaves receive less attention in scientific studies, despite the fact that people consume them frequently. Free radicals can be neutralised by *Amaranthus* antioxidant substances. Proteolytic enzymes originating from plants also aid in the metabolism of proteins and the production of physiologically active peptides that may influence inflammatory reactions.

The stability of plant bioactive chemicals can be greatly impacted by processing techniques like drying. Compared to dried leaves, fresh leaves may retain more enzymatic and antioxidant activity. With an emphasis on their significance in cancer-preventive nutrition, this study sought to assess the antioxidant capacity and proteolytic enzyme activity of fresh and dried *Amaranthus* leaves.

Material and Methods

Fresh leaves of *Amaranthus* species were obtained from the local market and washed thoroughly to remove any impurities. A portion of the leaves was analyzed fresh, and the rest was shade-dried at room temperature and ground into powder. Antioxidant activity was measured using the DPPH radical scavenging test. Both aqueous and methanolic extracts were prepared, and percentage inhibition was calculated. Proteolytic enzyme activity was measured using a tyrosine-based test and reported as µmol/g/hr. All experiments were done in triplicate, and the results were expressed as mean ± standard deviation.

Results and Discussion

Fresh *Amaranthus* leaf extracts showed greater antioxidant activity than dried extracts. The methanolic extracts demonstrated better extraction of phenolic substances, as indicated by their higher radical scavenging activity compared to aqueous extracts. Similar observations have been reported previously,[3-8] who attributed the antioxidant activity of *Amaranthus* leaves to the presence of phenolic compounds and betalains. Although *Amaranthus* exhibits lower antioxidant levels compared to *Moringa*, its activity remains nutritionally significant due to its frequent consumption as a common leafy vegetable.[2] Since oxidative stress plays a key role in cancer development, the antioxidant potential of *Amaranthus* may contribute to cancer prevention.

Table 1. DPPH Radical Scavenging Activity of *Amaranthus* Leaves.

Extract Type	Inhibition % Mean ± SD
Fresh – Aqueous	57.12 ± 3.23
Dried – Aqueous	48.29 ± 2.57
Fresh – Methanolic	62.86 ± 1.25
Dried – Methanolic	56.92 ± 1.67

Table 2. Proteolytic Enzyme Activity of *Amaranthus* Leaves.

Leaves	Protease Activity (µmol/g/hr) Mean ± SD
Fresh Leaves	33,600 ± 1,500
Dried Leaves	28,200 ± 1,200

Proteolytic enzyme activity was higher in fresh *Amaranthus* leaves compared to dried leaves, indicating partial loss of enzymatic activity during drying. Drying-related reduction in enzyme activity has been previously reported in leafy vegetables.[9] Proteolytic enzymes may support cancer-preventive mechanisms by aiding protein digestion and generating

bioactive peptides with anti-inflammatory properties. The observed reduction in activity in dried samples highlights the importance of consuming fresh leafy vegetables to retain enzymatic benefits.[10]

Conclusion

The current study shows that *Amaranthus* species have quantifiable proteolytic enzyme and antioxidant activity, with fresh leaves having higher bioactivity than dried leaves. Despite having less enzymatic and antioxidant activity than *Moringa oleifera*, *Amaranthus* is a valuable functional food due to its accessibility and regular dietary consumption. Including fresh *Amaranthus* leaves in daily diets may help lower inflammation and oxidative stress, which are linked to the development of cancer.

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Conflict of Interest

The author declares no conflict of interest.

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