IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# Evaluation Of Antioxidant Potential Of Leaf Extracts Of *Tinospora Formanii*

B. Suresh, S. Shabana, M. Satya prasad, M.R. Darukamalli, A. Krishna Satya*

Department of Biotechnology, Acharya Nagarjuna University,
Nagarjuna Nagar – 522 510, Guntur, Andhra Pradesh, India.

***Abstract:*** The present study deals with the antioxidant potential of *Tinospora formanii* leaf extracts. The leaves were collected and subjected to solvent extraction. Different solvent extracts (hexane, chloroform, methanol, water) were prepared, and their antioxidant activities were assessed using DPPH, FRAP, nitric oxide, hydroxyl radical, and hydrogen peroxide scavenging assays. Methanol extracts exhibited the highest yield and demonstrated strong antioxidant activity in most assays, with notable $IC_{50}$ values. The findings underline the significance of solvent polarity in extract yield and antioxidant potential. Methanol extracts showed superior scavenging activity, possibly due to their high phenolic content, making them promising sources of natural antioxidants. These results contribute to understanding the potential health benefits of *T. formanii* and its extracts in mitigating oxidative stress-related disorders.

***Index Terms*** *- Tinospora formanii*, leaf extracts, antioxidant potential.

## I. Introduction

Medicinal plants are central to traditional medical systems (Saleem et al., 2001). Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants accumulated over the many centuries is based on different medicinal systems, such as Ayurveda, Unani, and Siddha. During the last few decades, there has been an increase in the study of medicinal plants and their traditional use in different parts of the world. Herbal remedies are considered the oldest forms of health care known to mankind on this earth (Rahamtulla et al., 2020). These systems prioritize natural products to enhance human well-being. Notably, around 80% of people in developing nations rely on traditional medicine due to its effectiveness, cultural resonance, and limited side effects (Shrestha & Dhillion, 2003). Yet, the lack of documented evidence and quality control hampers the acceptance of alternative medicine. Establishing standards for medicinal plants is a pressing need. A valuable approach is employing techniques like pharmacognostic and phytochemical studies, contributing to plant identity standardization. These endeavors aid in characterizing and assuring the quality of herbal remedies, supporting their safety and efficacy validation (Odugbemi 2008). This study, therefore, delves into the assessment of the antioxidant activity of *Tinospora formanii.*

## II. Materials and Methods

*T. formanii* leaves were collected from the herbal garden of Acharya Nagarjuna University (Guntur, India) and shade-dried. The shade-dried leaf material was pulverized and powder was stored in airtight containers until use.

The dried leaf powder was extracted progressively (Wiart et al. 2004) using various solvents based on their polarities, such as hexane, chloroform, methanol, and water, using a Soxhlet apparatus (Lin et al. 1999). The extracts are concentrated and solvent-free at decreased pressure using a rotary evaporator. The dried crude concentrated extracts were weighed and kept in an airtight bottle until utilized for analysis to determine the extractive yield.

**Determination of antioxidant activity**

**DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay**

The antioxidant activity assessment involved preparing a 0.004% w/v DPPH solution in 95% ethanol as a radical source. Stock solutions of hexane, chloroform, methanol, water extracts, and ascorbic acid standard were prepared at 1mg/1 ml. These stocks were diluted to 100-500 µg/ml concentrations, mixed with DPPH solution, and incubated for 15 minutes. After measuring optical density at 523 nm, percent inhibition was calculated using the equation:

percent inhibition = ((OD Control - OD_Sample) / OD_Control) × 100.

The experiment was replicated thrice. The $IC_{50}$ value, indicative of the concentration necessary to neutralize 50% of DPPH free radicals, is ascertained by constructing a dose-response curve utilizing varying concentrations of the plant extracts (Rahamtulla et al., 2023).

**Ferrous reducing antioxidant power assay (Total antioxidant activity assay)**

Ferric reducing antioxidant power assay was carried out according to the modified protocol of Benzie & Strain (1996) method. In this method, different concentrations of hexane, chloroform, methanol, water extracts, and standard ascorbic acid were mixed with 1 ml of 0.2 M pH 6.6 sodium phosphate buffer and 1 ml of 1% potassium ferricyanide in separate tubes. Following a 20-minute incubation at 50°C in a controlled water bath, 1 ml of 10% trichloroacetic acid was introduced to each mixture. After centrifugation at room temperature for 10 minutes, the 1 ml supernatant was mixed with 1 ml deionized water and 200 µl of 0.1% FeCl3. A parallel blank was prepared, replacing potassium ferricyanide with distilled water. Measurement of the absorbance at 700 nm provided the basis for determining the increase in A700 after subtracting the blank, establishing the reducing power (Banerjee et al., 2008).

**Nitric oxide scavenging activity**

The nitric oxide scavenging activity was assessed using Garrat's (1964) method. Sodium nitroprusside in pH 7.4 phosphate buffer saline was incubated with hexane, chloroform, methanol, water extracts, and standard ascorbic acid at varying concentrations for 2 hours at 25°C. After incubation, the mixture was combined with sulfanilic acid reagent (33% in 20% glacial acetic acid) for 5 minutes at room temperature. Subsequently, naphthyl ethylenediamine dihydrochloride (0.1% w/v) and water were added, followed by a 30-minute incubation at room temperature. Measuring absorbance at 540 nm using a spectrophotometer allowed the determination of nitric oxide radical scavenging (Garrat, 1964; Nagulendran et al., 2007).

**Hydroxyl radical scavenging**

Hydroxyl radical scavenging was assessed using the 2-deoxyribose method by Halliwell et al. (1984). The test mixture contained 2-deoxyribose, ferric chloride, EDTA, and potassium phosphate buffer at various sample concentrations (100-500 µg/mL) at pH 7.4. Components are dissolved in 10 mM phosphate buffer. After incubating with an $H_2O_2$ and ascorbate solution, the reaction mixture was treated with TBA and TCA, followed by boiling. Absorbance at 532 nm measured 30% of the total reaction volume. Butylated hydroxytoluene (BHT) was used as a positive control. The inhibition percentage (I %) was calculated using absorbance values, and Ic50 values were determined by plotting the inhibition percentage against sample concentration, indicating the sample's hydroxyl radical scavenging potential.

**Hydrogen peroxide scavenging activity**

The hydrogen peroxide scavenging activity of *T. formanii* leaf extracts was assessed following the method by Ruch et al. (1989). Plant extracts (4 ml) were combined with a 4 mM $H_2O_2$ solution (0.6 ml) in a 0.1 M phosphate buffer (pH 7.4), and incubated at varying concentrations (100-500 µg/ml) in distilled water for 10 minutes. Absorbance was measured at 230 nm against a blank solution devoid of $H_2O_2$. The percentage scavenging activity was calculated as

((Abs. c - Abs. s) / Abs. c) × 100,

where Abs. c is the absorbance of the control and Abs. s is the absorbance of the sample. Ascorbic acid served as the positive control. All experiments were performed in triplicate.

## III. Results & Discussion

In the present study, *Tinospora formanii* extracts are analyzed for their physicochemical characteristics. A noticeable color variation in the extracts indicated the presence of diverse compounds in the solvent extracts, signifying the heterogeneous nature of the plant's constituents. Methanol leaf extract showed the highest percentage yield of extract (Fig. 1). This observation further supported the correlation between solvent polarity and the solubility of bioactive components (Sultana et al., 2009).

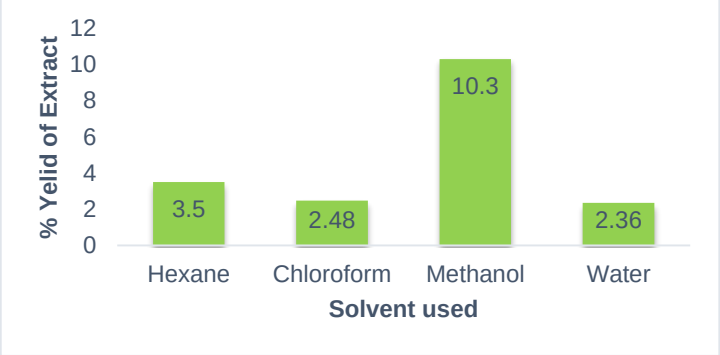


**Fig.1 DPPH radical scavenging activity**

The DPPH radical scavenging activity of *T. formanii* leaf extracts is shown in Figure 2. In the present investigation, dose-dependent inhibition was observed up to the given concentration; when the concentration of the extract was raised, the radical scavenging activity increased. Similar findings were reported in other medicinal plants concerning antioxidant efficacy (Sonter et al., 2021; Rahamtulla et al., 2023). Among the extracts tested, methanol extract had better scavenging activity ($IC_{50}$ value of 117.63±1.0 µg/ml) followed by hexane ($IC_{50}$ value of 222.73±1.5 µg/ml). When compared to Ascorbic acid which had an $IC_{50}$ value of 92.05±1.61 µg/ml. This highlights the link between phenolic compounds in methanol extracts of *T. formanii* leaves and their effective antioxidant action, consistent with prior research by Liu et al. (2008), Amensour et al. (2010), and Kuate et al. (2010) emphasizing the role of total phenolic content in enhancing antioxidant performance.

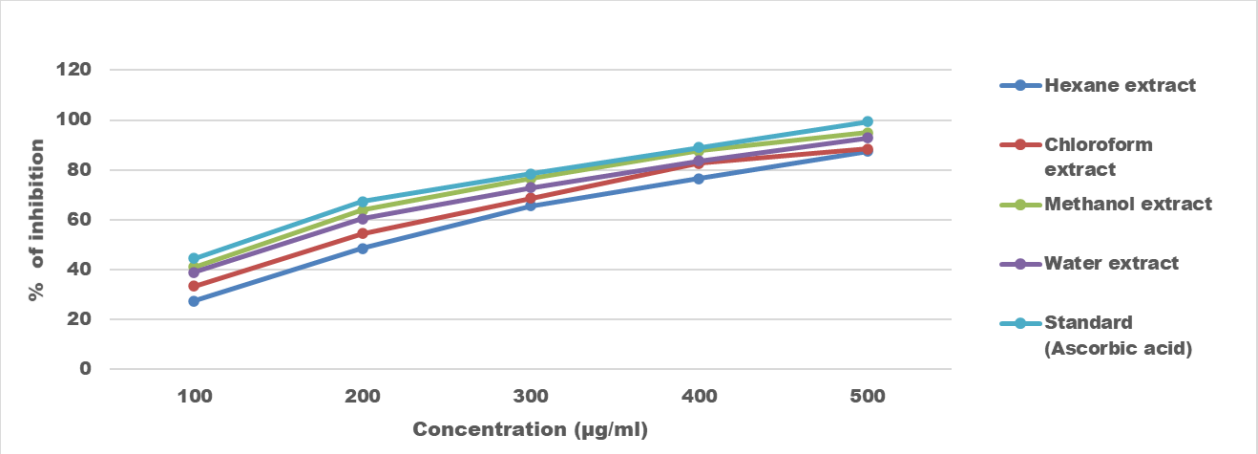


**Fig.2 DPPH Radical Scavenging Assay of *T. formanii* Leaf extract**

Ferric reducing antioxidant power (FRAP Assay)

The ability of the plant extracts to reduce ferric ions was determined by FRAP assay (Figure 3). An anti-oxidant capable of donating a single electron to the ferric-TPTZ (Fe (II)-TPTZ) complex would cause the reduction of the complex into the blue ferrous TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. The methanol leaf extract exhibited the showed good antioxidant activity i.e., the highest percentage of inhibition and $IC_{50}$ value (222.55±1.01 µg/ml)) while hexane leaf extracts showed $IC_{50}$ values (387.75±1.60 µg/ml), indicating lower antioxidant activity. This aligns with the reduction ability associated with antioxidant potential, supported by Meir et al. (1995). Our study found a positive relationship between increased antioxidant activity and higher polyphenol content, consistent with Oktay et al. (2003). Extract phenolic compounds served as reducing agents, hydrogen donors, and singlet oxygen quenchers, confirming

observations by Rice-Evans et al. (1997). These results underscore the antioxidant potential of these extracts, suggesting applications for combating oxidative stress and promoting health.

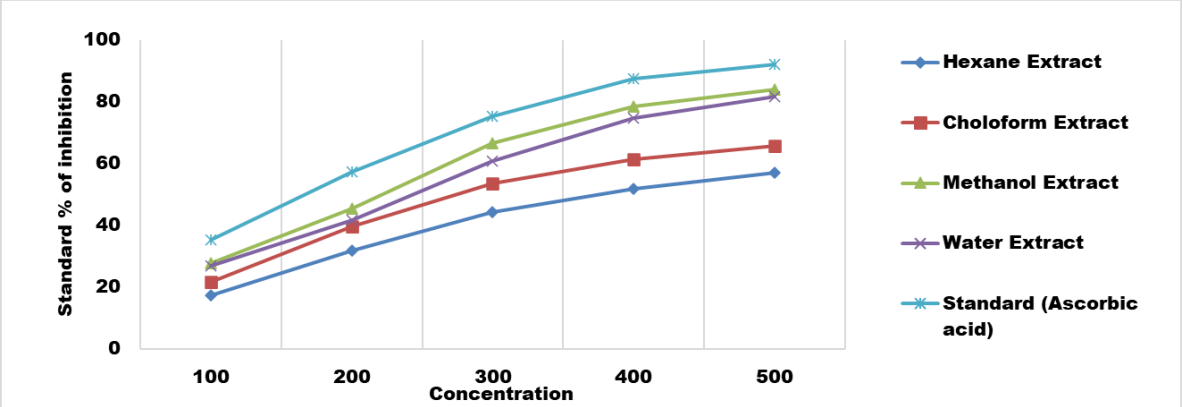


**Fig.3 Ferric reducing antioxidant power (FRAP Assay) of *Tinospora formanii* Leaf**

**Nitric oxide scavenging activity assay:**

The results showed that among the extracts tested, the methanol extracts from the leaves displayed the most robust nitric oxide scavenging activity. This was evident from their notably good $IC_{50}$ values of 88.96±1.51 µg/ml, indicating a high efficiency in neutralizing nitric oxide radicals (Fig. 4). Conversely, the hexane extracts from the leaves exhibited moderate $IC_{50}$ values, indicating a comparatively weaker ability to scavenge nitric oxide, with values of 174.20±1.05 µg/ml.

The study examined nitric oxide scavenging in *T. formanii* leaf extracts compared to Ascorbic acid. Methanol extracts displayed robust activity with good $IC_{50}$ values (88.96±1.51 µg/ml), surpassing Ascorbic acid. These findings suggest potent natural antioxidants in the extracts, particularly methanol ones. This highlights their potential as alternatives to synthetic antioxidants for combating oxidative stress-related diseases and supporting health.

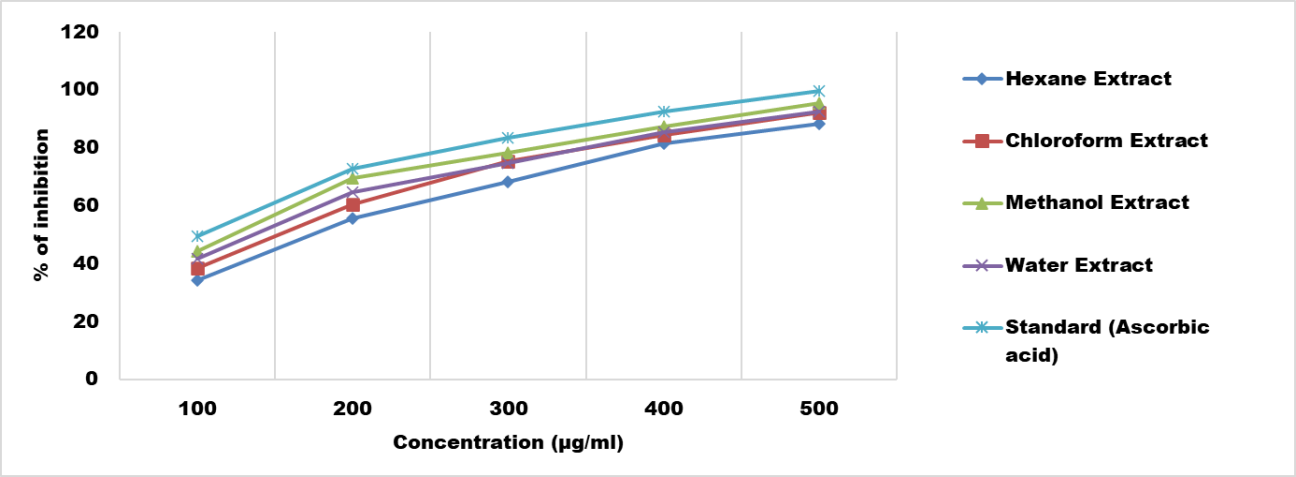


**Fig.4 Nitric oxide Scavenging activity assay of *Tinospora formanii* Leaf extract**

**Hydroxyl Radical Scavenging Activity**.

Among *T. formanii* leaf extracts, the methanol extract exhibited the highest hydroxyl radical scavenging activity with a good $IC_{50}$ value. The $IC_{50}$ value signifies the concentration needed for a 50% reduction in initial scavenging activity, reflecting extract potency as hydroxyl radical scavengers. Specifically, the methanol leaf extract displayed good $IC_{50}$ values (69.11±2.62 µg/ml), while hexane leaf extracts had moderate IC50 values (158.65±0.99 µg/ml), indicating lower hydroxyl radical scavenging (Fig. 5).

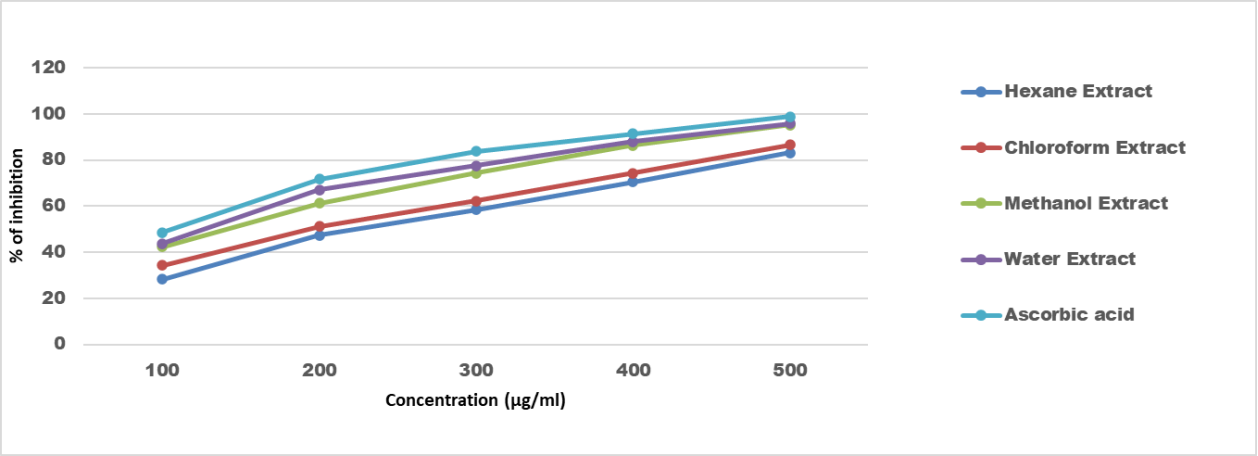


**Fig.5 Hydroxyl Radical Scavenging Activity of *Tinospora formanii* Leaf extract**

## Hydrogen peroxide scavenging activity

*T. formanii* leaf extracts were tested for their ability to scavenge hydrogen peroxide ($H_2O_2$) radicals. The most potent scavenging was observed in methanol extracts, with an $IC_{50}$ of 121.91±0.76 µg/ml, followed by water and chloroform extracts (Fig. 6). Hexane extracts exhibited the weakest scavenging. The presence of phenolic compounds in the extracts contributes to effective electron donation, converting $H_2O_2$ into water. This demonstrates strong antioxidant activity across all extracts, swiftly transforming $H_2O_2$ into water through efficient electron donation.

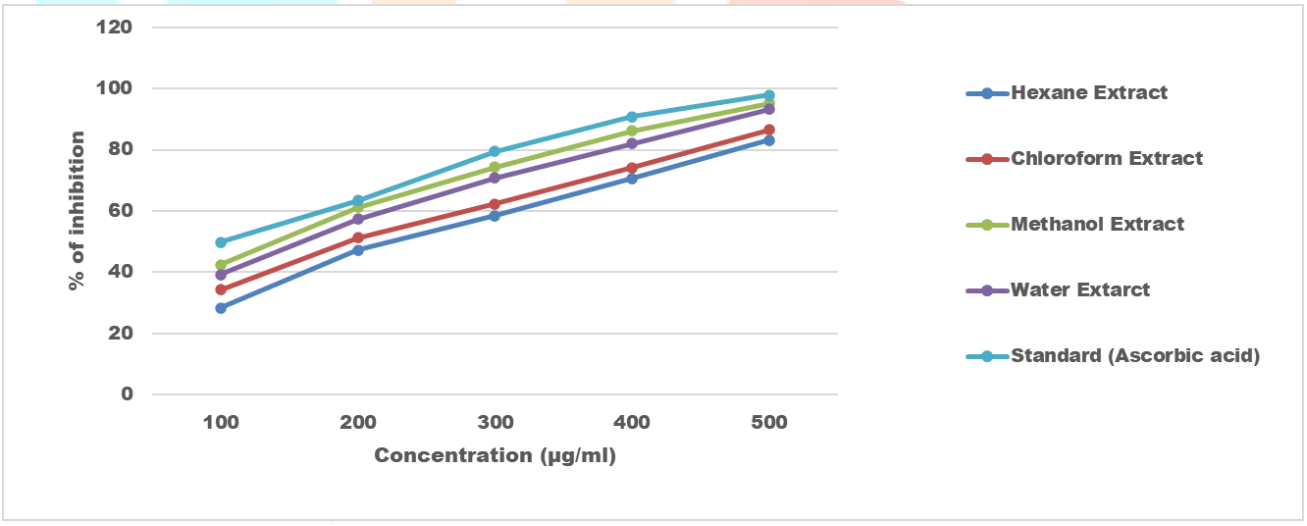


**Fig.6 Hydrogen peroxide scavenging activity of *Tinospora formanii* Leaf**

## IV. Conclusion

In the present study, the methanol leaf extract of *T. formanii* showed good antioxidant activities when compared to the other solvent extracts. This might be due to the high phenolic and flavonoid content of the extract. It may be beneficial to investigate the leaf extract further for its potential applications in oxidative stress-induced diseases.

## V. References


[1] Amensour, M., Sendra, E., Pérez-Alvarez, J. A., Skali-Senhaji, N., Abrini, J., & Fernández-López, J. (2010). Antioxidant activity and chemical content of methanol and ethanol extracts from leaves of rockrose (Cistus ladaniferus). *Plant foods for human nutrition*, *65*, 170-178.

[2] Banerjee, R., Phan, A., Wang, B., Knobler, C., Furukawa, H., O'Keeffe, M., & Yaghi, O. M. (2008). High-throughput synthesis of zeolitic imidazolate frameworks and application to CO2 capture. *Science*, *319*(5865), 939-943.

[3] Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

[4] Garrat, D. C. (1964). The quantitative analyze of drugs. Japan.

[5] Halliwell, B., & Gutteridge, J. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical journal*, *219*(1), 1.

[6] Kuate, D., Etoundi, B. C. O., Soukontoua, Y. B., Ngondi, J. L., & Oben, J. E. (2010). Antioxidant characteristics of Dichrostachys glomerata spice extracts Caracteristicas antioxidantes de los extractos de la especia Dichrostachys glomerata. *CyTA–Journal of Food*, *8*(1), 23-37.

[7] Lin, X., Buff, E. M., Perrimon, N., & Michelson, A. M. (1999). Heparan sulfate proteoglycans are essential for FGF receptor signaling during Drosophila embryonic development. *Development*, *126*(17), 3715-3723.

[8] Liu, L. (2008). BEST: Bayesian estimation of species trees under the coalescent model. *Bioinformatics*, *24*(21), 2542-2543.

[9] Meir, S., Kanner, J., Akiri, B., & Philosoph-Hadas, S. (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of agricultural and food chemistry*, *43*(7), 1813-1819.

[10] Nagulendran, K. R., Velavan, S., Mahesh, R., & Begum, V. H. (2007). In vitro antioxidant activity and total polyphenolic content of Cyperus rotundus rhizomes. *Journal of Chemistry*, *4*, 440-449.

[11] Odugbemi, T. (Ed.). (2008). *A textbook of medicinal plants from Nigeria*. Tolu Odugbemi.

[12] Oktay, M., Gülçin, İ., & Küfrevioğlu, Ö. İ. (2003). Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. *LWT-Food Science and Technology*, *36*(2), 263-271.

[13] Rahamtulla, M., Mallikarjuna, K., & Khasim, S. M. (2023). GC-MS analysis and therapeutic importance of leaf extracts of *Dendrobium aphyllum* (Roxb.) CEC Fischer: An In vitro study. *South African Journal of Botany*, *153*, 62-76.

[14] Rahamtulla, M., Pradhan, U. C., Roy, A. K., Rampilla, V., & Khasim, S. M. (2020). Ethnomedicinal aspects of some orchids from Darjeeling Himalaya, India. *Orchid biology: Recent trends & challenges*, 451-472.

[15] Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.

[16] Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10(6), 1003-1008.

[17] Saleem, A., Ahotupa, M., & Pihlaja, K. (2001). Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan. *Zeitschrift für Naturforschung C*, *56*(11-12), 973-978.

[18] Shrestha, P. M., & Dhillion, S. S. (2003). Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. *Journal of ethnopharmacology*, *86*(1), 81-96.

[19] Sonter, S., Mishra, S., Dwivedi, M. K., & Singh, P. K. (2021). Chemical profiling, in vitro antioxidant, membrane stabilizing and antimicrobial properties of wild growing *Murraya paniculata* from Amarkantak (MP). *Scientific Reports*, *11*(1), 9691.

[20] Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, *14*(6), 2167-2180.

[21] Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M., ... & Sulaiman, M. (2004). Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia*, *75*(1), 68-73.