# Comparative Study of Antioxidant Potential of *Colebrookea oppositifolia* Root and Aerial Parts

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**Abstract:** Oxidative stress is an important factor contributing to the aetiology of many degenerative diseases and is brought on by an imbalance between pro-oxidants and antioxidants in an organism. Free radicals have the ability to alter DNA as well as biomolecules like protein, lipids, polyunsaturated fatty acids, carbohydrates, and nucleic acids. The oxidation of biomolecules can be effectively delayed by antioxidants. Food-derived antioxidants can delay or stop oxidation by preventing the initiation and dissemination of oxidizing chain reactions. Since ancient times, medicinal plants have been used to treat illnesses because plants contain several kinds of antioxidant compounds like flavonoids, phenolics, thiol and steroids. Colebrookea oppositifolia Sm. (CO) traditionally has been used in treatment of various disorders associated with stress. In this study, the radical scavenging abilities of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals were compared to L-ascorbic acid, which served as the standard, in order to determine the in vitro antioxidant activity of plant extract. In addition to that total phenolic content and total flavonoid content were also estimated. The obtained results revealed that roots of plant exhibited strong antioxidant properties than the aerial parts and the study recommend that root extract may be used in In-vivo studies against associated oxidative stress-mediated complications.

**Keywords:** Anti-oxidant, *Colebrookea oppositifolia* Sm., DPPH, $H_2O_2$, total flavonoid content, Total phenolic content

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**Abbrevations:**

CO: *Colebrookea oppositifolia* Sm., DPPH: 1,1-diphenyl-2-picrylhydrazyl; $H_2O_2$: Hydrogen peroxide; MACO: Methanolic Aerial extract of *Colebrookea oppositifolia* Sm., MRCO:

Methanolic roots extract of *Colebrookea oppositifolia* Sm., ROS: Reactive oxygen species; TFC: Total flavonoid content.

**Introduction**: Reactive oxygen species (ROS), such as hydroxyl radical, single oxygen, hydrogen peroxide radical, superoxide anion, hydroxyl radicals, singlet oxygen, hypochlorite radicals and nitric oxide radicals cause damage to a number of physiological systems in the human body. Exogenous variables such as ionizing radiation, smoking, pollution, pesticides, and organic solvent can cause oxidative stress, which can damage protein, enzymes and nucleic acids. In normal physiological condition human body have equilibrium state of free radical and antioxidants. But in pathological condition level of ROS increases and to restore physiological condition demands of antioxidants increased (Mau *et al*., 2002; Adebayo *et al*., 2012).

Antioxidants are molecules that neutralizes ROS and stop them to cause cell damage. They are mainly used in small quantities to retard the process of oxidation of products like lipids which can be easily oxidised. In plants, antioxidants primarily serve as scavengers and aid in the transformation of radicals into less reactive species (Balmus *et al*., 2016). Antioxidants block the process of oxidation by neutralizing free radicals. Plants have anti-oxidant characteristics which are primarily responsible for their traditional usage as a remedy for some diseases where oxidative damage is suspected. Plants have antioxidant qualities because they include phenolic chemicals including flavonoids, tannins, and terpenoids that can provide free radical scavenging action (Miliauskas *et al*., 2014).

The ability of polyphenols to chelate metals, block or diminish a variety of enzymes like lipoxygenase, telomerase, cyclooxygenase, and scavenge free radicals are all indicators of their medicinal usefulness. The bulk of polyphenolic substances with antioxidant activity have many functions and work primarily through these pathways (Yin *et al*., 2008; Kortei *et al*., 2014). Furthermore, it is well understood that a molecule's antioxidant properties are dependent on the extraction method and compound complexity, necessitating the use of multiple techniques to assess its antioxidant activity (Gioti *et al*., 2009).

*Colebrookea oppositifolia* Sm. (CO), is a branched shrub, commonly known as Indian Squirrel Tail. It usually grow upto 1-3 m long at an altitude of 250–1700 m (Yadav *et al*., 2021). It is belonging to Lamiaceae family and widely distributed in India, Nepal, China, Myanmar, Pakistan, and Thailand. It has been used for medicinal, ethnic, and spiritual reasons for a long time. Indian systems of medicine, Traditional Chinese Medicine and Ayurveda utilize the roots, aerial parts, and leaves of this plant for their potential impact on lipid peroxide content, memory, anti-aging and anti-cholinergic actions. It has been widely utilized to treat a variety of disorders including traumatic brain injury, rheumatoid arthritis, stroke, and epilepsy (Chopra *et al*., 1956; Ishtiaq *et al*., 2016). So, the current study was designed to evaluate the antioxidant activity of MACO and MRCO using the DPPH scavenging assay, hydrogen peroxide test, total flavonoid and phenolic content estimation.

**Procedure and Experimentation:**

**Chemicals and Reagents:** Catechin, gallic acid, ascorbic acid, aluminium chloride, sodium phosphate, sodium nitrite, hydrogen peroxide, 1,1-diphenyl-2-picrylhydrazyl, folin-ciocalteus`s phenol reagents used were of analytical grade procured from Sigma Aldrich.

**Collection and extraction of plant material**

CO aerial and root parts were collected in September 2022 from the highlands of Mukteshwar, Nainital, India. Dr. S.S Yadav, Associate Professor, Department of Botany, Maharshi Dayanand University, Rohtak, Haryana, validated the plant. Specimen voucher (MDU/BOT/1209). The shade dried aerial part and roots of CO were successively extracted in a Soxhlet extractor at 40° C temperature using methanol solvent. The extracts obtained were filtered and then concentrated with the help of rotary evaporator under reduced pressure. After that concentrated/ semisolid extracts were lyophilized and stored in an airtight container at cool place.

**Antioxidant Activity of the Plant Extracts**

- **2,2-diphenyl-2-picryl-hydrazyl radical scavenging (DPPH) Assay**:

The extracts' ability to scavenge free radicals was evaluated using the DPPH radical scavenging assay. The decolorization of a methanol solution of DPPH was used to evaluate the plant extract's capability to donate hydrogen atoms. It produces a purple coloration in methanol solution that fades to a yellow colour in the presence of an antioxidant (Sethi *et al*., 2020; Thaipong *et al*., 2006). Plant extract stock solution was made by dissolving extract in methanol at a concentration of 1mg/ml. Diluted concentrations of 100, 200, 300, 400, and 500 g/ml were produced from the stock solution. In the same way, a standard solution of ascorbic acid was made in the same concentration range. In methanol, a 0.135 mM solution of DPPH was prepared. 1 ml of DPPH and 1 ml of different concentration of extract was taken. The mixture was vigorously shake and kept in a dark room for 30 minutes. The absorbance was taken spectrophotometrically at 517 nm. Blank was prepared without addition of sample. Absorbance of plant extract is compared with the standard solution (ascorbic acid) concentration.

Using following formula, inhibition percentage calculated

$$\textbf{Inhibition Percentage (\%)} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ was absorbance of DPPH + methanol

$A_{sample}$ was absorbance of DPPH + sample (i.e. standard or extract)

- **Hydrogen peroxide ($H_2O_2$) radical scavenging activity**

The traditional approach for determining hydrogen peroxide (H2O2) scavenging activity of plant extract is to measure the disappearance of H2O2 at 230 nm. Several oxidase enzymes in the body produce hydrogen peroxide in vivo. Hydroxide peroxide scavenges by producing a hydroxyl radical as a reduction product. When scavenger is incubated with hydrogen peroxide in this approach, the decay and loss of hydrogen peroxides are detected spectrophotometrically at 230 nm. Decolorization of phosphate buffer solution of H2O2 was used to test the antioxidant activity of plant extract (Klein *et al*., 1981).

The stock solution of 1 mg/ml of plant extract was prepared in distilled water. Different

concentrations of extract were prepared 100, 200, 300, 400, 500 µg/ml using stock solution. Standard (Ascorbic acid) concentration was prepared in similar manner. In phosphate buffer solution (50 mM pH - 7.4), hydrogen peroxide (40 mM) is prepared.

1ml of extract and 0.6 ml of hydrogen peroxide was taken and mixed properly, after 10 min absorbance was taken at 230 nm. Blank solution contained phosphate buffer instead of extract or standard solution (Khan *et al*., 2013).

The percentage of hydrogen peroxide scavenging activity measured by using following formula

$$\textbf{Inhibition Percentage (\%)} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ was absorbance of control

$A_{sample}$ was absorbance of sample/standard.

- **Total phenolic content:**

Plant secondary metabolites are phenolic compounds. These molecules have an aromatic ring with one or more hydroxyl groups and can be as simple as a phenolic molecule or as complex as a high-molecular-weight polymer. The number and position of the hydroxyl groups, as well as the type of aromatic ring modification, all influence the antioxidant activity of a phenolic molecule. Phenolic compounds have the ability to directly scavenge a variety of reactive species, such as peroxyl, hydroxyl and superoxide radicals, in order to perform their antioxidant activity. Some phenolic compounds can bind metals that promote oxidation, such as iron and copper, preventing the formation of free radicals.

The total phenolic content of CO's aqueous and ethanolic extracts was determined using the Folin-Ciocalteau reagent (Do *et al*., 2014; Youn *et al*., 2019). The calibration curve (Figure 1) was constructed by combining 1 ml aliquots of 20, 40, 60, 80, 100, and 120 mg/ml Gallic acid solutions with 5.0 ml of tenfold-diluted Folin Ciocalteu reagent and 4.0 ml of sodium carbonate solution (75 g/l). After 30 minutes, the absorbance was measured at 765 nm. To make the calibration curve, 1 ml of each of the aqueous and ethanolic extracts (1 gm/100 ml) was mixed with the same reagents separately. After one hour, the absorbance was measured in order to compute the total phenolic content in each extract individually using the following formula:

$$C = C_1 + V/M$$

C = total phenolic content in mg/g in gallic acid equivalent

$C_1$ = Concentration of gallic acid.

V = Volume of extract in ml

W-sample weight in gram.

In particular, they are a category of secondary plant metabolites having a polyphenolic structure that are present in fruits, vegetables, and some drinks. Flavonoids constitute a vast group of natural compounds. They possess numerous advantageous biochemical and antioxidant properties that have been linked to a number of diseases, including Alzheimer's disease, cancer, and atherosclerosis (Chang *et al*., 2002; Park et al., 2008). Total flavonoid content is a measure of the quantity of flavonoids present in a sample. It can be determined using various chemical methods, including colorimetry, spectrophotometry, and high-performance liquid chromatography.

The total flavonoid contents of the MRCO and MACO were estimated using the aluminum chloride colorimetric assay (Fattahi *et al*.,2014). The calibration curve (Figure 2) was plotted by mixing 0.5 ml aliquots of 20, 40, 60, and 80 mg/ml rutin solutions with 0.5 ml of 2% aluminum chloride solution. The absorbance was measured after one hour to calculate the total flavonoid content in each extract individually using the procedure.

$$C = C_1 + V/M$$

C = total flavonoid content in mg/g in rutin equivalent

$C_1$ = Concentration of rutin.

V = Volume of extract in ml

W-sample weight in gram.

**Statistical analysis:**

GraphPad Prism 5.0 software was used to conduct all analyses in triplicate, and the results were presented as mean standard error of the mean (S.E.M.). Analysis was done on the comparison of MRCO and MACO at various concentrations.

**Results:**

**Percentage Yield:** percentage yield of aerial and root parts of CO is as shown in table 1. Percentage yield is high in root extract compared to aerial parts.

**Table 1: Percentage Yield of CO**

| Plant material | Weight of plant material | Solvent used | Percentage yield (w/w) |
|---|---|---|---|
| Aerial part | 500g | Methanol | 14.8 |
| Roots | 500g | Methanol | 20.4 |

**Total Phenolic content:**

The type of sample, solvent, and temperature employed for extraction all affect the polyphenol content. According to Table 2, the phenolic content of MRCO was higher than that of MACO by 12.50. Figure 1 exhibits a standard curve for the total phenolic content.

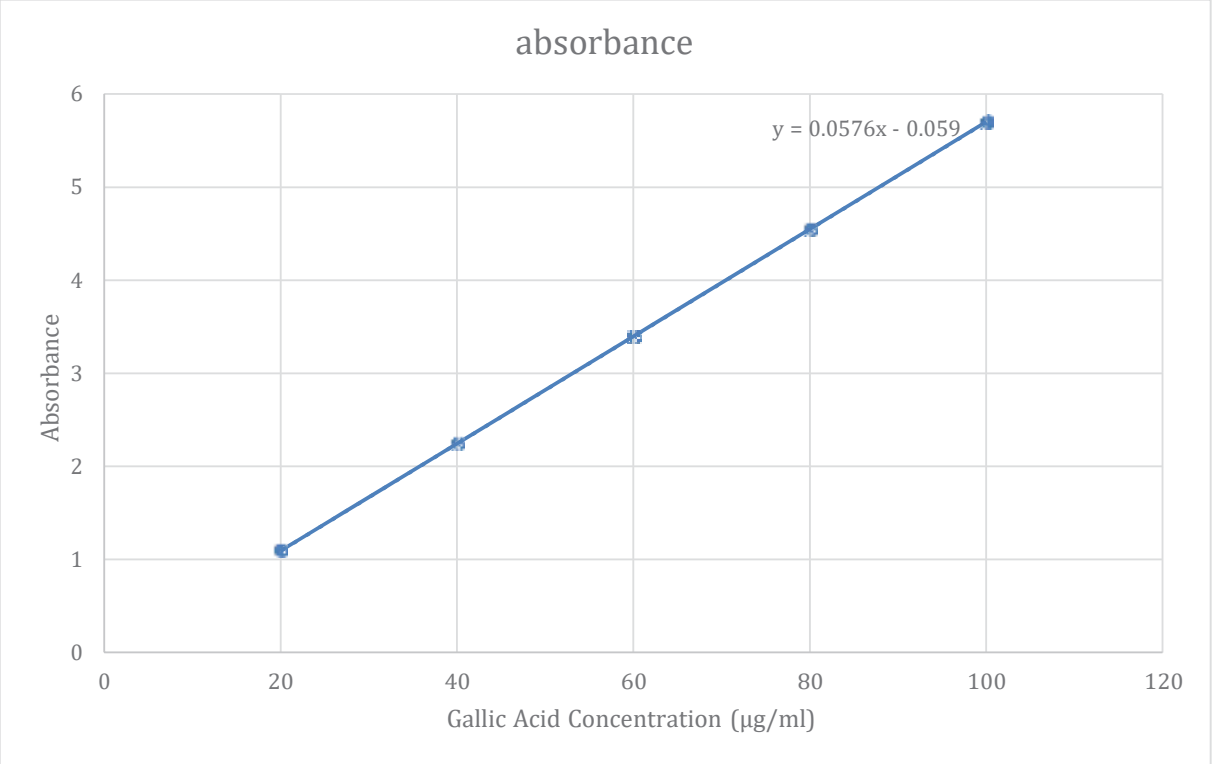


**Figure 1: Standard curve for total phenolic content**

**Table 2: Total Phenolic Content of MRCO and MACO**

| **Extract** | **Phenolic Content (mg GAE/g dry wt.)** |
|---|---|
| MRCO | 39.75 |
| MACO | 27.25 |

**Total Flavonoid Content:** The quantity of polyphenols present in the extract, as well as the extraction solvent and temperature, all affect the flavonoid content. Table 3 indicates that MRCO had a greater flavonoid content than MRCO. Figure 2 illustrates a standard curve for the total flavonoid content.

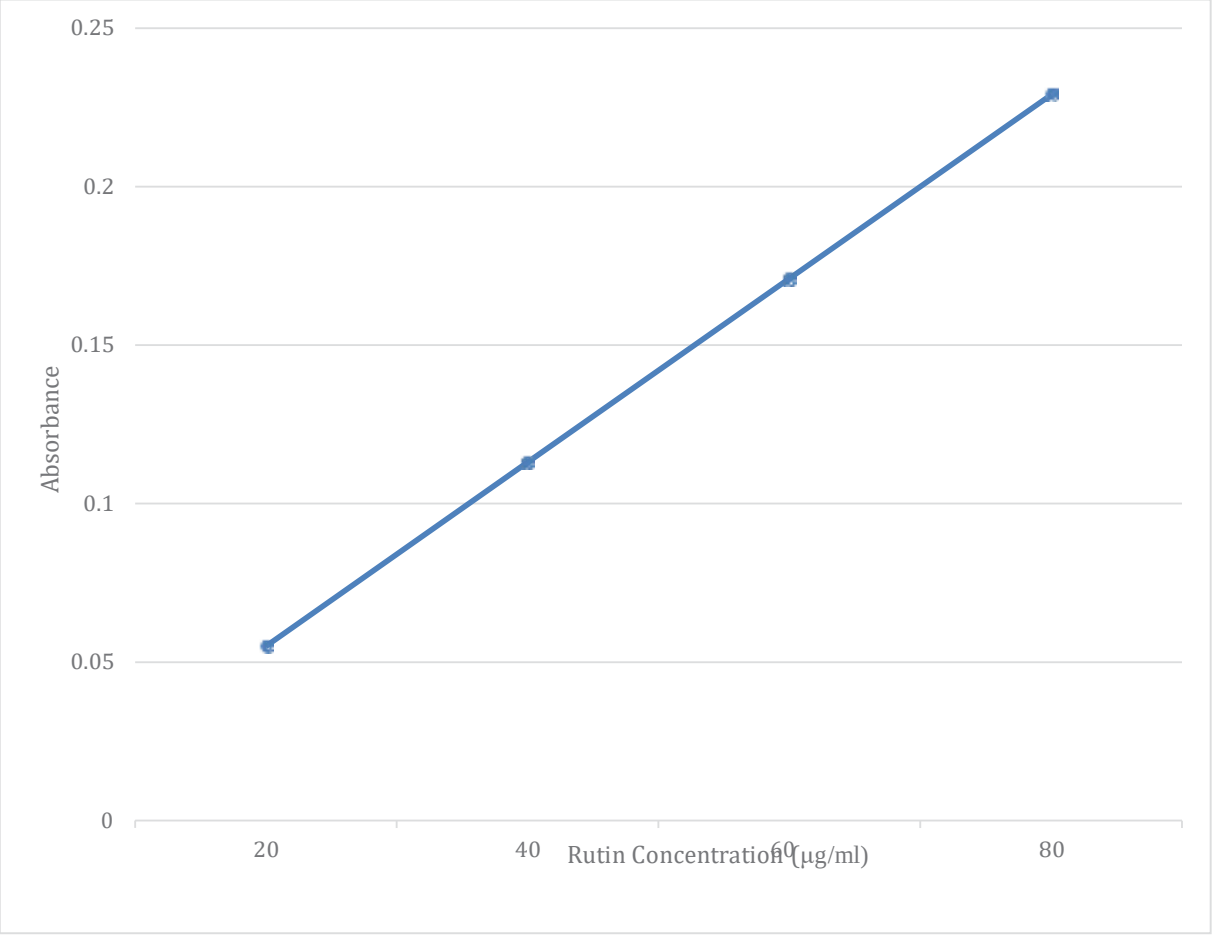


**Figure 2: Standard curve for total flavonoid content**

**Table 3: Total Flavonoid Content of MRCO and MACO**

| **Extract** | **Flavonoid Content (mg RE/g dry wt.)** |
|---|---|
| MRCO | 16.86 |
| MACO | 9.8 |

**Hydrogen Peroxide Assay:**

The MRCO and MACO demonstrated remarkable scavenging activity in a dose dependent manner as shown in table. The standard (Ascorbic acid) exhibited significantly higher antioxidant activity ($P < 0.05$). At all the studied concentration, MRCO exhibited higher antioxidant activity as compared to MACO as shown in Table 4 and figure 3.

**Table 4: $H_2O_2$ scavenging activity of Ascorbic acid, MRCO and MACO**

| | **% Inhibition** | | |
|---|---|---|---|
| **Concentration** | **Ascorbic Acid** | **MRCO** | **MACO** |
| 100 | 36.49±0.058 | 30.96±0.033 | 26.79 ± 0.041 |

| | | | |
|---|---|---|---|
| 200 | 46.99 ± 0.037 | 40.67 ± 0.043 | 33.55 ± 0.034 |
| 300 | 59.01±0.037 | 49.10 ± 0.051 | 40.52 ± 0.084 |
| 400 | 66.49 ± 0.058 | 57.44 ± 0.095 | 49.62 ± 0.064 |
| 500 | 72.72 ± 0.057 | 66.20 ± 0.076 | 57.29 ± 0.092 |

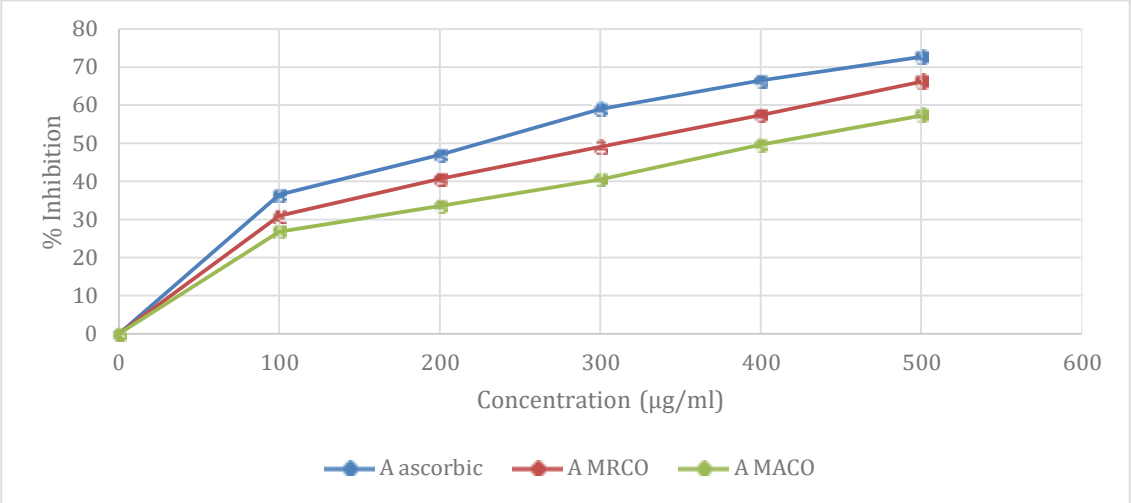


**Figure 3: $H_2O_2$ scavenging activity of Ascorbic acid, MRCO and MACO**

**DPPH Assay:**

In a dose-dependent manner, MRCO and MACO exhibited significant DPPH radical scavenging activity. As can be seen in table 5 and figure 4, the standard ascorbic acid had the highest scavenging activity, followed by MRCO, and then MACO.

**Table 5: DPPH scavenging activity of Ascorbic acid, MRCO and MACO**

| **Concentration** | **% Inhibition** | | |
|---|---|---|---|
| | **Ascorbic acid** | **MRCO** | **MACO** |
| 100 | 26.6±0.55 | 19.56±0.41 | 13.77±0.41 |
| 200 | 37.08±0.49 | 31.61±0.38 | 20.65±0.54 |
| 300 | 49.6±0.38 | 42.41±0.44 | 36.93±0.56 |
| 400 | 60.56±0.65 | 55.24±0.49 | 51.95±0.41 |
| 500 | 69.79±0.28 | 61.97±0.27 | 56.96±0.61 |

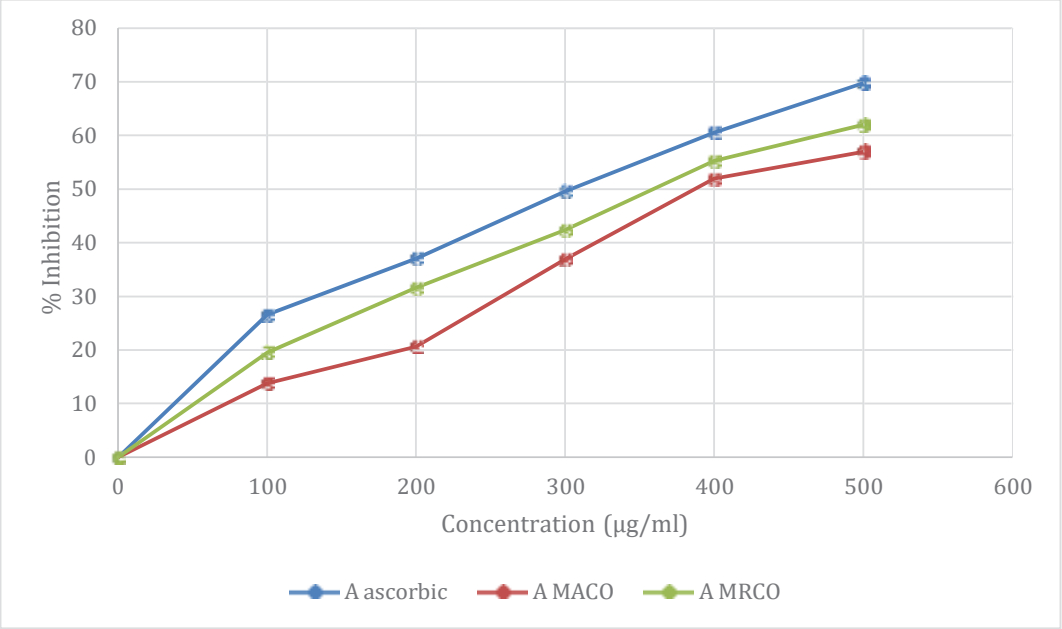


**Figure 4: DPPH scavenging activity of Ascorbic acid, MRCO and MACO**

**Discussion:** The present study investigated the potential antioxidant activity of MACO and MRCO. Antioxidants are compounds that inhibit the oxidation of other molecules and can protect cells from oxidative stress. Plants are a rich source of natural antioxidants, including flavonoids and phenolic compounds. The results of this study revealed that the MRCO exhibited stronger antioxidant activity compared to the MACO. This finding is consistent with previous studies that have reported the antioxidant activity of other plants' root extracts, which are often rich in phenolic and flavonoid compounds (Benzie *et al.*,1996; Xu *et al.*, 2007). The higher antioxidant activity of the root extract could be attributed to the higher content of phenolic and flavonoid compounds found in this extract. While flavonoids have been shown to have a wide range of biological activities, such as anti-inflammatory, antioxidant, and anticancer effects, phenolic compounds are well known for their capacity to scavenge free radicals and inhibit lipid peroxidation. The higher content of these compounds in the root extract suggests that this part of the plant could be a more potent source of natural antioxidants. It is worth noting that the total flavonoid and phenolic content of both extracts was relatively high, indicating that CO is a rich source of these compounds. It is well recognized that flavonoids and phenolic compounds have a number of health advantages, including lowering the risk of chronic illnesses like cancer, diabetes, and cardiovascular disease. Therefore, the consumption of plant-based foods and supplements rich in these compounds may have potential health benefits. Overall, the results of this study suggest that MRCO could be a promising source of natural antioxidants with potential health benefits. Further studies are needed to explore the active compounds responsible for the observed antioxidant activity and their potential medicinal applications.

**Conclusion:** In conclusion, this study investigated the MRCO and MACO for their in vitro antioxidant activity and total flavonoid and phenolic content. The results showed that the root extract exhibited stronger antioxidant activity compared to the aerial part extract. This was attributed to the higher content of phenolic and flavonoid compounds in the root extract. These results indicate that MRCO may be a source of natural antioxidants with health benefits. Further studies are needed to explore the potential medicinal applications of this plant and its active compounds.

**References**

Adebayo, E.A., Oloke, J.K., Ayandele, A.A. and Adegunlola, C.O., 2012. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from Pleurotus pulmonarius-LAU 09 (JF736658). Journal of Microbiology and Biotechnology Research, 2(2), pp.366-374.

Balmus, I.M., Ciobica, A., Trifan, A. and Stanciu, C., 2016. The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association, 22(1), p.3.

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

Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis, 10(3).

Chopra, RN., Nayar, SL., Chopra IC., 1995. Glossary of Indian Medicinal Plants Council of Scientific and Industrial Research. New Delhi. 89.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H., 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of food and drug analysis, 22(3), pp.296-302.

Fattahi, S., Zabihi, E., Abedian, Z., Pourbagher, R., Ardekani, A.M., Mostafazadeh, A. and Akhavan-Niaki, H., 2014. Total phenolic and flavonoid contents of aqueous extract of stinging nettle and in vitro antiproliferative effect on hela and BT-474 Cell lines. International journal of molecular and cellular medicine, 3(2), p.102.

Gioti, E.M., Fiamegos, Y.C., Skalkos, D.C. and Stalikas, C.D., 2009. Antioxidant activity and bioactive components of the aerial parts of Hypericum perforatum L. from Epirus, Greece. Food Chemistry, 117(3), pp.398-404.

Ishtiaq, S., Meo, M.B., Afridi, M.S.K., Akbar, S. and Rasool, S., 2016. Pharmacognostic studies of aerial parts of Colebrookea oppositifolia Sm. Ann Phytomed, 5(2), pp.161-167.

Khan, H.A.A., Shad, S.A. and Akram, W., 2013. Resistance to new chemical insecticides in the house fly, Musca domestica L., from dairies in Punjab, Pakistan. Parasitology research, 112, pp.2049-2054.

Klein, S.M., Cohen, G. and Cederbaum, A.I., 1981. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry, 20(21), pp.6006-6012.

Kortei, N.K., Odamtten, G.T., Obodai, M., Appiah, V., Akuamoa, F., Adu-Bobi, A.K., Annan, S.N.Y., Armah, J.N.O. and Acquah, S.A., 2014. Evaluating the effect of gamma radiation on the total phenolic content, flavonoids, and antioxidant activity of dried Pleurotus ostreatus ((Jacq. ex. Fr) Kummer) stored in packaging materials. Advances in Pharmaceutics, 2014.

Mau, J.L., Lin, H.C. and Song, S.F., 2002. Antioxidant properties of several specialty mushrooms. *Food research international*, *35*(6), pp.519-526.

Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry, 85(2), pp.231-237.

Park, H.H., Lee, S., Son, H.Y., Park, S.B., Kim, M.S., Choi, E.J., Singh, T.S., Ha, J.H., Lee, M.G., Kim, J.E. and Hyun, M.C., 2008. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. Archives of pharmacal research, 31, pp.1303-1311.

Sethi, S., Joshi, A., Arora, B., Bhowmik, A., Sharma, R.R. and Kumar, P., 2020. Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. European Food Research and Technology, 246, pp.591-598.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of food composition and analysis, 19(6-7), pp.669-675.

Xu, B.J. and Chang, S.K.C., 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of food science, 72(2), pp.S159-S166.

Yadav, D.K., 2019. Pharmacognostical, phytochemical and pharmacological profile of Colebrookea oppositifolia Smith. Journal of Drug Delivery and Therapeutics, 9(6-s), pp.233-237.

Yin, J., Heo, S.I. and Wang, M.H., 2008. Antioxidant and antidiabetic activities of extracts from Cirsium japonicum roots. Nutrition research and practice, 2(4), pp.247-251.

Youn, J.S., Kim, Y.J., Na, H.J., Jung, H.R., Song, C.K., Kang, S.Y. and Kim, J.Y., 2019. Antioxidant activity and contents of leaf extracts obtained from Dendropanax morbifera LEV are dependent on the collecting season and extraction conditions. Food science and biotechnology, 28, pp.201-207.