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Research Article

# *In vitro* Antioxidant Activity and Total Phenolic Content of *Monstera deliciosa.*

Pal Prosanta[1*] Chakraborty Mainak[2], Karmakar Indrajit[2], Haldar Sagnik[2], Das Avratanu[2], Haldar Pallab Kanti[2]

[1]*Himalayan Pharmacy Institute. Majhitar, East Sikkim 737136, Sikkim.*
[2]*Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, West Bengal, India*

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**ABSTRACT**
Objectives: The present study evaluates the free radical scavenging activity and total phenolic content of methanol extract of *Monstera deliciosa*. Methodology: The antioxidant activities of above mentioned extract of *Monstera deliciosa* were measured by different *in vitro* standard methods like 1,1-diphenyl-2-picrylhydrazil radical (DPPH), nitric oxide, superoxide anions, hydroxyl radicals. On the basis of antioxidant properties total phenol content was estimated for methanol extract of *Monstera deliciosa* (MEMD). Results: The extracts exhibited antioxidant activities in a dose dependent manner. The $IC_{50}$ values of the Methanol extract of *Monstera deliciosa* and that of the standard (ascorbic acid) for DPPH are 77.87± 2.21 µg/mL, 20.73± 1.18 µg/ml ; for Nitric oxide are 81.30 ± 1.19 µg/mL, 33.53 ± 1.81 µg/mL; for Superoxide are 85.33 ± 3.18 µg/mL, 25.33 ± 2.33 µg/mL, for Hydroxyl radical are 89.33± 1.76 µg/mL, 39.33± 2.40 µg/mL .Conclusion: The study elucidated that MEMD possesses significant dose dependant antioxidant activity. Further research is going on to find out the active principle(s) of MEMD responsible for its antioxidant activity.

**Key Words:** *Monstera deliciosa*, Antioxidant, 1,1-diphenyl-2-picrylhydrazil radical, Free radicals.

## INTRODUCTION

Plant and its products are rich sources of a phytochemical and have been found to possess a variety of biological activities including antioxidant potential[1].We are interested to find out the antioxidant property of *Monstera deliciosa* which is commonly used in folk medicine. The plant *Monstera deliciosa* is a large climber, native of central America , cultivated in gardens throughout India for its large, bright green, perforated leaves and cone-like fruits[2]. The fruits have a mixed flavour of pineapple and banana and are considered a delicacy. The remnants of the flowers are said to create irritation of the throat. Consumption of the fruit also causes some times allergy or anaphylaxis[3]. The seeds are strong purgative, leaves used by Chinese for treating some cancers[4]. In Mexico, a leaf or root infusion is drunk daily to relieve arthritis[2]. The root is used to make a remedy for snakebite[3].

Free Radicals are molecules with an unpaired electron. Due to the presence of a free electron, these molecules are highly reactive. They are important intermediates in natural processes involved in cytotoxicity, control of vascular tone, neurotransmission and other diseases[5].

The body also has systems to repair or replace damaged building blocks of cells. Most protein constituents in the cell are completely replaced every few days. Scavenger enzymes break used and damaged proteins into their component parts for reuse by the cell. Vitamins and other nutrients neutralize the oxy radicals' and serves as second line of defence. Among the many substances used are Vitamins C and E, beta-carotene, and bioflavonoids[6]. Antioxidants act as free radical scavengers and are thus found to play significant protective role against oxidative stress in a variety of diseases such as liver cirrhosis [7], inflammation, atherosclerosis[8], diabetes, cancer[9], neurodegenerative disease[10], nephrotoxicity and also the aging process [11].

## MATERIALS AND METHODS

*Plant material*

The leaves of *Monstera deliciosa* was collected from Majhitar village of East Sikkim and identified by Department of Pharmacognosy, Himalayan Pharmacy Institute East Sikkim, India. The leaves were washed thoroughly with water then dried in shade. The shade dried leaves (800 gms) were powdered in a mechanical grinder and the powdered materials was extracted successively with petroleum ether, chloroform and methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary
vacuum evaporator. The concentrated extracts were stored in vacuum desiccators for further use.

*Chemicals*

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate(PMS), reduced nicotinamide adenine dinucleotide (NADH),sodium

*Author for Correspondence

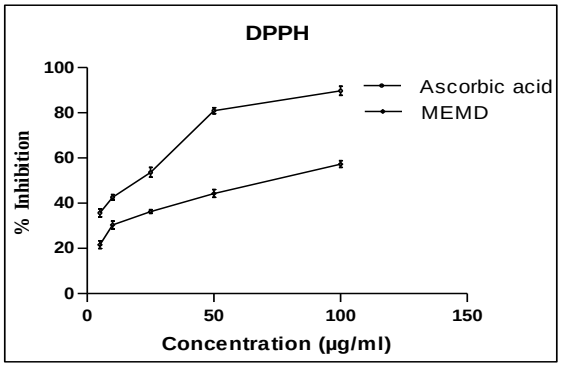


Figure1: 1,1-diphenyl-2-picrylhydrazil (DPPH) scavenging activity of MEMD and the standard ascorbic acid. The data represent the percentage of DPPH inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM)

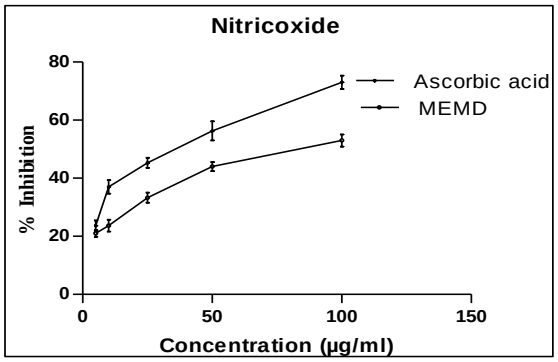


Figure.2: The nitric oxide radical scavenging activity of MEMD and standard ascorbic acid. The data represent the percentage of nitric oxide inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM)

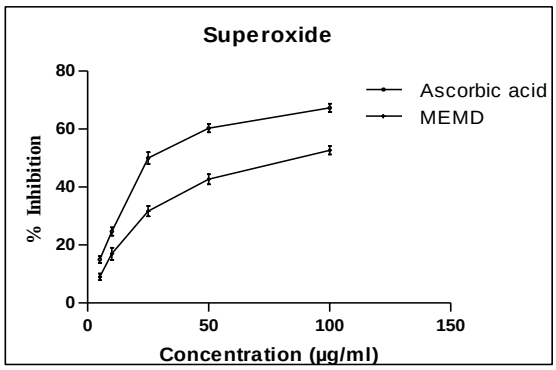


Figure. 3: Superoxide radical scavenging assay of MEMD and the standard ascorbic acid. The data represent the percentage of superoxide inhibition. Each point represents the values obtained from three experiments, performed in triplicate (mean ± SEM)

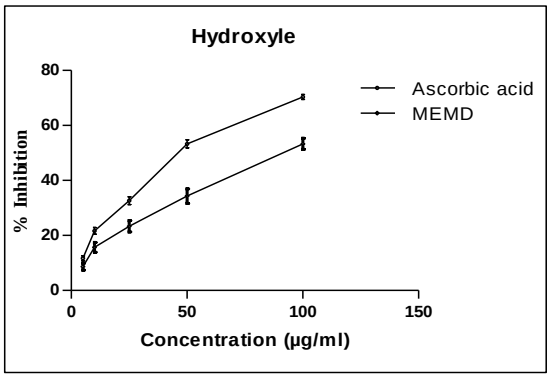


Figure.4: The hydroxyl radical scavenging activity of MEMD and standard ascorbic acid. The data represent the percentage of OH inhibition. Each point represents the values obtained from three experiments, performed triplicate(mean ± SEM)

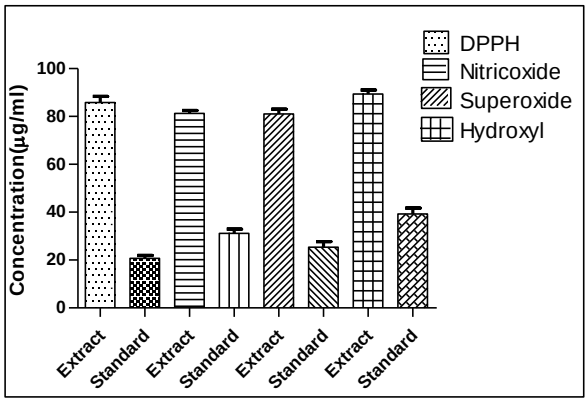


Figure. 5: $IC_{50}$ values. The $IC_{50}$ values of the *Monstera deliciosa* for DPPH, nitric oxide, superoxide, hydroxyl radical scavenging activity are 77.87±2.21µg/ml and 81.30 ± 1.19 µg/ml, 85.33 ± 3.18 µg/ml, and 89.33± 1.76 µg/ml. All the values are mean ±SEM ($n$ = 3).

nitroprusside, napthyl ethylene diamine dihydrochloride, ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA), sodium hydroxide (NaOH), hydrogen peroxide (H2O2) butylated hydroxy anisole (BHA), deoxyribose, potassium ferricyanide [K3Fe(CN)6] , Folin-Ciocalteu's phenol reagent (FCR) were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals were used in high analytical grade.

*DPPH radical scavenging activity*

DPPH radical scavenging activity was measured using the described method Cotelle *et al*. 1996[12] with some modifications by Tamilarasan *et al* 2015[13].The percentage inhibition was calculated by using the following formula.

Percentage inhibition = [(C – T)/C] x 100
Where,
C = Absorbance at 517 nm of the control and
T = Absorbance at 517 nm of the test [12].

*Nitric oxide*

The nitric oxide radicals scavenging activity was determined as per previously describe method by Subramaniyan *et al* 2014.[14]

*Superoxide radical scavenging activity*

The super oxide radicals scavenging activity was determined as per previously describe method by Bala *et al* 2012.[15]

*Hydroxyl radical scavenging activity*

The hydroxyl radicals scavenging activity was determined as per previously describe method by Karmakar *et al* 2011[13].

*Determination of total phenolic content*

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent according to the Stanojevic *et al* 2009[16].

*Statistical analysis*

All the data are given as the mean ± SEM. $p < 0.05$ was considered significant.

**RESULTS**

The leaf of *M deliciosa* extract shows a remarkable capacity to scavenge all the tested reactive species and all the $IC_{50}$ values (mean+ SEM) for three individual experiments found at µg /ml.

In vitro antioxidant assay

From the $IC_{50}$ value of the *M deliciosa* extract (77.87± 2.21 µg/ml) in comparison to the reference ascorbic acid (20.73± 1.18 µg /ml) to scavenge the DPPH radical, it can be put forward as a fact that the extract truly work as antioxidant (Figure.1 and 5).

Figure. 2 showed the dose dependent inhibition on nitric oxide of extract and standard ascorbic acid. The $IC_{50}$ value of extract and ascorbic acid were found to be 81.30 ± 1.19 µg/ml and 33.53 ± 1.81 µg/ml (Figure. 5) respectively.

Superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The increase in inhibition capability indicates the extract has good superoxide radicals scavenging activity as compared to standard ascorbic acid (Figure. 3). The $IC_{50}$ values of extract and ascorbic acid were found to be 85.33 ± 3.18 µg/ml and 25.33 ± 2.33 µg/ml (Figure. 5) respectively.

The extract showed potent hydroxyl radical scavenging activity as compared to standard ascorbic acid (Figure. 4). The $IC_{50}$ value of the extract and standard in this assay were found to be 89.33± 1.76 µg/ml and 39.33± 2.40 µg/ml (Figure. 5) respectively.

Total phenolic content was determined using the Folin-Ciocalteu's (FC) reagent and it was calculated as 27.50 µg/mg of *M deliciosa* extract which is accounted for its free radical as well as antioxidant activity.

**DISCUSSION**

Oxidative stress refers to a situation where in the production of oxidants exceeds the capacity to neutralize them, leading to damage to cell membranes, lipids, nucleic acids, proteins and constituents of the extracellular matrix such as proteoglycans and collagens[17]. Different therapeutic approaches can be used to decrease the oxidative stress and include scavenging of free radicals, inhibition of free radical producing enzymes, enhancing the antioxidant system or by targeting the signaling routes and expression of molecules involved in the inflammatory cascade. Amongst the intracellular ROS generated, the superoxide plays a pivotal role in inflammation[18].

DPPH is a stable free radical, which has been widely used in phytomedicine for the assessment of scavenging activities of bioactive fractions.

In its radical form, DPPH absorbs at 517 nm, but upon re duction with an antioxidant, its absorption decreases

Due to the formation of it's non-radical form. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. Figure 1 shows free radical scavenging. The scavenging activities of MEMD was determined using free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH). Results showed that MEMD ($IC_{50}$ 77.87 ± 2.21 µg/ml) possessed the good antioxidant activity as compared to standard[19].

In reaction mixture sodium nitropruside react with oxygen to from nitric oxide which measured spectrophotometrically at 546 nm. Nitric oxide is recognized to be an inter and intra cellular mediator of several cell functions. It acts as a signal molecule in immune, nervous and vascular systems. The toxicity increases greatly when it forming the highly reactive peroxynitrite anion ($ONOO^-$) The present study proved that the extract has good nitric oxide scavenging activity (Figure 2)[15].

Superoxide radical is highly toxic species, which is generated by numerous biological reactions. Plant products are rich sources of flavonoid and phenolic compound which are effective antioxidant mainly because of their scavenging property. The results suggest that concentration-dependent increasing of superoxide radical scavenging activity. PMS-NADH coupling release superoxide which can be measured by their ability to reduce NBT. The increase in inhibition capability indicates the extract has good superoxide radicals scavenging activity (Figure 3). The IC50 values of extract and ascorbic acid were found to be 85.33± 3.18 µg/ml and 25.33±2.33 µg/ml (Figure 5) respectively[19].

Hydroxyl radical reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. Pathophysiology of various disease hydroxyl radical plays a crucial role and it can able to damage almost every molecule in biological system leads to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly proportional to its antioxidant activity which is depicted by the low intensity of pink colour [20].

Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food. Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified [21]. The plant contains 25.7 µg of phenol compound in 1000µgm of the methanol extract. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability.

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