Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN 0976-9595
Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *IPOMOEA MARGINATA* LEAVES AND WHOLE PLANT

P.N. Sukitha[1], G. Uma*[2], T. Citarasu[2], P. Jini[1]
[1]*Department of Biotechnology, Noorul Islam College of Arts &Science, Kumaracoil, Kanyakumari, TamilNadu, India*
[2]*Center for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari, TamilNadu, India*
**Corresponding author: umaganapathi23@gmail.com*

Received: 21-03-2022; Revised: 03-06-2022; Accepted: 09-06-2022; Published: 30-06-2022
 https://doi.org/10.55218/JASR.202213506

ABSTRACT
The present study was aimed to study enzymatic antioxidants (superoxide dismutase, catalase, polyphenol oxidase), non-enzymatic antioxidants (saponin, flavonoids, ascorbic acid and tannin), and anti-oxidant ability assays (total anti-oxidant, hydroxyl radical scavenging ability, reducing power activity, inhibition of lipid peroxide formation, DPPH scavenging activity and metal chelating assay) in *I. marginata* leaves and whole plant.
The results revealed that *I. marginata* possessed significant enzymatic anti-oxidants activities like superoxide dismutase, catalase, polyphenol oxidase in whole plant of *I. marginata*. The non-enzyme anti-oxidants namely saponin, flavonoids, ascorbic acid and tannin showed maximum activity in leaves while the total phenol in whole plant. The anti-oxidant ability assays such as reducing power activity, DPPH scavenging activity and metal chelating assay were found to be high in whole plant. From the study, it was confirmed that the whole plant of *I. marginata* showed strong antioxidant ability assay. Thus *I. marginata* whole plant can be used as a potential source of natural anti-oxidants.

Keywords: *Ipomoea marginata,* Enzymatic antioxidants, Antioxidant ability assays, Non-enzymatic antioxidants.

1. INTRODUCTION
India is the land of medicinal plants and it is important to register the data by scientific researches on such plants could be of clinical importance. Treating various health issues like cancer, arthritis and diabetes has been documented in Ayurvedic medical system practiced mainly in the Asian continent more than 5000 years [1]. Plant is an important source of medicine and plays a key role in world health [2]. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. Medicinal plants contain various ranges of chemical molecules with pharmacological applications. In recent years botanists, ethanopharmacologist and natural product chemist are analyzing the available medicinal plants for extracting various phytochemicals in the light of emerging various drug resistance fungi and bacteria [3]. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions.
Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Free radicals are generated during this oxidative reaction especially during oxidative respiration. When there is a mitochondrial leakage of activated oxygen which in turn initiate a chain of reactions that results in cellular damage [4]. Antioxidants terminate this chain of reactions by removing free radical intermediates thus inhibiting further oxidation reaction [5]. To balance the oxidative stress, plants and animals maintain complex systems of overlapping antioxidants such as glutathione and enzymes (*e.g.*, catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C and vitamin E.
Antioxidants are a class of secondary metabolites of plant. The plant kingdom offers many polyphenolic compounds. Several isolated plant constituents as well as extracts have possessed antioxidant effects against free radicals in biological systems. Natural antioxidants have a wide range of biochemical activities including inhibition of ROS generation, direct or indirect scavenging of free

radicals and alteration of intracellular redox potential. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases. In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases [6]. It is by now commonly accepted that under situations of oxidative stress, reactive oxygen species such as superoxide (O_2^-), hydroxyl (OH^-), and peroxyl (^-OOH, ROO^-) radicals are generated. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts, and inflammation. The use of traditional medicine is widespread, and plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepato-protective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity. In searching for novel natural antioxidants, some plants have been extensively studied in the past few years for their antioxidant and radical scavenging components [7]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. A variety of free radical scavenging antioxidants exists within the body of which many are derived from dietary sources like fruits, vegetables and teas [8].

The genus Ipomoea has been used since ancient time has been in incessant use for various purposes, such as food, vegetable, medic ornamentals, and hallucinogens and in religious rituals. At present, due to advancement of techniques in horticulture, floristic and genetics, many new hybrid varieties are formed, advances in biochemical tools has made possible to identify and isolate bioactive compounds from these plants. As genus Ipomoea is rich in phytochemicals, many medicinally important species have been worked out for their active ingredients but many of them are still to be discovered. At present, the species of genus Ipomoea are used in different ways as described here [9].

Ipomoea is the largest genus in the flowering plant family Convolvulaceae, with over 500 species. The generic name is derived from the Greek words meaning "resembling". It refers to their twining habit. The genus occurs throughout the tropical and subtropical regions of the world. Humans use *Ipomoea* for their content of medical and psychoactive compounds, mainly alkaloids. The genus includes food crops; the tubers of sweet potatoes and the leaves of water spinach are commercially important food items. The various species have wide medical applications. They are used to treat blood disease, sterility in women, urinary infection, constipation, gynecological disorder. The plant is also having laxative, psychedelic, anticarcinogenic, hepato-protectivity, oxytocic, and antioxidant properties. They are also used in rheumatism and fungal infection [10].

The aim of the present study is to investigate the antioxidant potential and radical scavenging activity of *I. marginata* using different *in vitro* assays, along with the determination of various enzymatic and non enzymatic antioxidants.

2. MATERIAL AND METHODS

2.1. Collection of Plant materials

Medicinally important plant species, *I. marginata*, belongs-to-family convolvulaceae was selected for the present investigation. The whole plants with the roots of *I. marginata* were collected from Karungal, Kanyakumari District, Tamilnadu. It was identified and authenticated by a taxonomist. Withered leaves and whole plant of *I. marginata* were rinsed under running tap water to eliminate the dust. After that the samples were washed several times with distilled water and air dried. The dried samples were powdered and the powdered samples were kept in a clean, dried, air tight glass container to protect it from sunlight [11].

2.2. Enzymatic antioxidants

2.2.1. Assay of Superoxide Dismutase (SOD)

A 1.4 ml of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer pH 7.4, 0.075 ml of 20 mM L-methionine, 0.04 ml of 1% Triton X 100, 0.075 ml of 10 mM hydroxylamine hydrochloride and 0.1 ml of 50 mM EDTA) was added to 100 µl of the sample extract and incubated at 30°C for 5 minutes. 80 µl of 50 µM riboflavin was added and the tubes were exposed for 10 minutes in the fluorescent lamps. After the exposure time, 1 ml of Greiss reagent (mixture of equal volume of 1% sulphanilimide in 5% phosphoric acid) was added and the absorbance of the colour formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions [12].

2.2.2. Assay of Catalase (CAT)

The enzyme extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.01 M phosphate buffer (pH

7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of μmoles of H_2O_2 consumed/min/mg protein [13].

2.2.3. Assay of Polyphenol Oxidase (PPO)

To 2.0 ml of plant extract and 3.0 ml of distilled water, 1.0 ml of cathecol solution (0.4 mg/ml) was added and the reactions mixture was quickly mixed. The enzyme activity was measured as change in absorbance/min at 490 nm [14].

2.2.4. Assay of Peroxidase

The reaction mixture consisted of 3 ml of pyrogallol (0.05 M pyrogall in 0.1 M phosphate buffer (pH 7.0) and 0.1ml of plant extract and the O.D. change was measured at 430 nm for every 30 seconds for 2 minutes [15].

2.3. Non-Enzymatic Antioxidants

2.3.1. Determination of Flavonoids

Five gm of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper no. 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed [16].

2.3.2. Determination of Total Phenols

For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were left for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm [17].

2.3.3. Determination of Saponin

Twenty gm of each sample were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethylether was added and shaken vigorously. The aqueous layer was recovered while ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight [17].

2.3.4. Determination of Ascorbic Acid

A 5 gm of the sample was weighed and 100 ml of EDTA/TCA (2:1) extracting solution was added, mixed well and the mixture was shaken for 30 min. This was then transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 min. It was transferred into a 100 ml volumetric flask and made upto 100 ml mark with the extracting solution. 20 ml of the extract was pipetted into a volumetric flask, 1% starch indicator was added and titrated with 20% $CuSO_4$ solution to get a dark end point [18].

2.3.5. Determination of Tannins

A 500 mg of the sample was weighed and 50 ml of distilled water was added and stirred for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$. The absorbance was measured at spectrophotometer at 395nm within 10 min [19].

2.4. Antioxidant and Free Radical Scavenging Activity

2.4.1. Hydroxide Radical-scavenging ability

Freshly prepared aqueous extract (0-100 ml) was added to a reaction mixture containing 120 ml of 20 mM deoxyribose, 400 ml of 0.1 M phosphate buffer, 40 ml of 20 mM hydrogen peroxide and 40 ml of 500 mM $FeSO_4$, and the volume was made with 800 ml with distilled water. The reaction mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid (TCA); this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in spectrophotometer [20].

2.4.2. Reducing property

A 2.5 ml of the plant extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, and then 2.5 ml of 10% TCA was added. This was then centrifuged at 650 rpm for 10 minutes. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm, and a higher absorbance indicated a greater reducing power [21].

2.4.3. Total Antioxidant Assay

Standard gallic acid solution or the extract (1 ml) was combined with 3.0 ml of the reagent solution containing 0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mM ammonium molybdate. The mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the solution was measured at 725 nm. Preparation of the calibration curve for total antioxidant capacity was carried out using gallic acid [22].

2.4.4. Inhibition of Lipid Peroxide Formation

Egg homogenate (0.5 ml of 10% v/v) and 0.1 ml of plant extracts were added to a test tube and made up to 1 ml with distilled water and peroxidation was induced by adding 0.05 ml of 0.07 M $FeSO_4$. The reaction mixture was then incubated for 30 minutes. 1.5 ml of 0.8% (w/v) TBA in 1.1% SDS and 20% TCA were added and the resulting mixture was vortexed and heated at 95°C for 60 minutes. After cooling, 5 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 minutes. The absorbance of the upper organic layer was measured at 532 nm [23]. The percentage of inhibition (I) was calculated by the formula:

$I = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$

2.4.5. DPPH Scavenging Assay

An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control [24]. DPPH free radical scavenging ability (%) was calculated by using the formula.

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

2.4.6. Metal chelating assay

Five ml of the plant extract was added to a solution of 0.1 ml of 2 mM $FeCl_2$. This was followed by the addition of 0.2ml of 5 mM ferrozine solution, which was left to react at room temperature for 10minutes under shaking conditions before determining the absorbance of the solution at 562 nm [25]. The percentage inhibition of ferrozine Fe^{2+} complex formation was calculated by using this formula:

$I = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$

3. RESULTS AND DISSCUSSION

The enzymatic profile of *I. marginata* seems to be good and the result was presented in the table 1. The levels of enzymatic antioxidant activity such as superoxide dismutase, catalase, polyphenol oxidase, and peroxidise values were tabulated in table 1.

Table 1: Enzymatic antioxidants of *I. marginata*

Parameters	IMW	IPL
Superoxide dismutase (unit/mg protein)	1.75±0.2746	1.748±0.3521
Catalase (µ moles of H_2O_2 consumed / min / mg protein)	30.724±0.1823	17.872±0.3658
Polyphenol oxidase (µ moles / g tissues)	43±14.4049	35±15.8114
Peroxidase (µ moles / g-sample)	1.606±0.1111	1.65±0.1623

Values are expressed as mean±SD (n = 5), IMW: I. marginata whole plant IML: I. marginata leaves

The whole plant of *I. marginata* showed higher activity of super oxide dismutase and the SOD levels in leaves are also somewhat similar to the whole plant. The catalase activity and polyphenol oxidase was higher in whole plant. Peroxidase activity was found to be high in leaves. From the study, it was confirmed that whole plants of *I. marginata* was rich in enzymatic antioxidnats.

The non-enzymatic antioxidants such as saponin, flavonoids, total phenol, ascorbic acid and tannin present in the whole plant and leaves of *I. marginata* was given in the table 2. Saponin, flavonoids, ascorbic acid and tannin content were high in the leaves whereas total phenol was high in the whole plant of *I. marginata*. From the study it was found that the leaves are the excellent sources of non-enzymatic antioxidants.

The antioxidant ability assays such as total antioxidant, hydroxide radical scavenging, reducing power activity, inhibition of lipid peroxide formation, DPPH

scavenging and metal chelation were determined in the whole plant and leaves of *I. marginata* and results were tabulated in the table 3. The assays such as total antioxidant, hydroxyl radical scavenging, and inhibition of lipid peroxide formation were rich in leaves of *I. marginata*. The reducing power activity, DPPH scavenging assay and metal chelation assay were high in whole plants of *I. marginata*.

Table 2: Non-enzymatic antioxidants of *I. marginata*

S. No.	Parameters	IMW	IPL
1.	Saponin (g/100 g)	3.704±0.1213	5.372±0.1970
2.	Flavonoids (g/100 g)	11.086±0.2050	20.144±0.2881
3.	Total Phenol (mg/g)	0.656±0.4559	0.344±0.1527
4.	Ascorbic Acid (g/100 g)	77.492±21.7395	102.294±32.4699
5.	Tannin (mg/100 g)	14.34±0.7635	23.6±0.2

Values are expressed as mean±SD (n = 5), IMW: I. marginata whole plant IML: I. marginata leaves

Table 3: Antioxidant ability assays of *I. marginata*

Parameters	IMW	IML
Total antioxidant assay (mg/dl)	1.3±0.0316	40±3.1623
Hydroxyl radical scavenging ability assay (mg/dl)	69.9±1.2942	74.2±1.5248
Reducing power activity (%)	40±7.9057	30.3±3.0331
Inhibition of lipid peroxide formation (%)	30.2±1.6047	80.3±1.8574
DPPH scavenging assay (%)	80.26±3.1623	60.52±3.1623
Metal chelation assay (%)	76.006±2.4091	67.268±1.5061

Values are expressed as mean±SD (n = 5), IMW: I. marginata whole plant IML: I. marginata leaves

Quantitative determination of total phenols and flavonoids in dry leaves, stem and flowers of *I. carnea* was done using spectrophotometric methods [26]. Kano et al., [27]; Prasad et al., [28] found that *Ipomoea* species such as *I. batata* and leaf extract of *I. aquatica* showed antioxidant properties. Yadav et al., [29] screened the antioxidant activities of DPPH free radical scavenging assay and reducing power assay of free radical scavenging activities *Artocarpus heterophyllus*. Daddala et al., [30] determined the DPPH radical activity of methanol extract of *Linum usitatisimum*. Sukitha et al., [11] studied the phytochemicals in the different extracts of *I. marginata* leaves and whole plants and found that alkaloids, carbohydrates, flavonoids, phenolic compounds, resins, saponins, steroids, tannins, terpenoids, protein, cardiac glycosides, reducing sugars, proteins and volatile oils showed the presence of alkaloids, carbohydrates, flavonoids, phenol compounds, steroids, tannins, terpenoids, protein and volatile oils were present in the leaves of whole plants of *I. marginata*. The antioxidant effect of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes. Phenolics are the largest group of phytochemicals and have been accounting for most of the antioxidant activity of plants or plant products. From the present study it was found that as a whole, both the whole plants and leaves of *I. marginata* contain promising amount of antioxidants.

4. CONCLUSION

The present study confirmed that the leaves and the whole plant of *I. marginata* showed promising antioxidant and radical scavenging activities and the difference in their antioxidant activities can be attributed to their difference in phenolic content. From the observations it can be concluded that the *I. marginata* act as a good source of natural antioxidant and might be useful in treating the diseases associated with oxidative stress. Further research is necessary to find the relation between antioxidants and oxidative stress in this plant *I. marginata*.

Conflict of interest
None declared

Source of funding
None declared

5. REFERENCES

1. Dahanukar SA, Kulkarni RA, Rege NN. *Ind J Pharmacol*, 2000; **32:**S81-S118.

2. Sadhana AS, Rao AR, Kucheria K, Bijani V. *Cancer Lett*, 1988; **40(2)**:193-197.
3. Selvakumar TA, Rajasekar P. *J Taiwan Inst Chem Eng*, 2017; **78**:539-551.
4. Okoli JTN, Agbo-MO, Ukekwe IF. Jor J Biol Sci, 2014:**7(4)**:251-235.
5. Aja PM,Odeh CO,Uraku AJ,Offur CE. *Int J Curr Microbial App Sci*, 2015; **4**:382-393.
6. Adsul VB, Eliza Khatiwora E, Deshpande NR. *J Nat Prod Plant Resour*, 2012; **2(5)**:584-588.
7. Dong-Jiann H, Hsien-Jung C, Chun-Der L, Yaw-Huei L. *Bot Bull Acad Sin*, 2005; **46**:99-106.
8. Lobo V, Patil A, Phatak A, Chandra N. *Pharmacogn Rev*, 2010; **4(8)**:118-126.
9. Deepa S. *Res J Recen Sci*, 2017; **6(10)**: 23-26.
10. Arora S, Kumar D, Shiba. *Int J Pharm Pharm Sci*, 2013; **5(1)**:198-202.
11. Sukitha PN, Uma G, Citarasu T, Jini P. *J Chem Pharm Res*, 2016; 8(1S):70-77.
12. Das K, Samanta K, Chainy GBN. *Ind J Biochem Biophys*, 2000; **37**:201-204.
13. Sinha AK. *Anal Biochem*, 1972; **47**:389-394.
14. Sadasivam S, Manickam A. 1996. Biochemical method: Polyphenol oxidase. 2nd ed. New Delhi: New Age International (P) Limited, 1996; p. 108-110.
15. Addy SK, Goodman RN. *Ind Phytopath*, 1972; **25**:575-579.
16. Boham AB, Kocipai-Abyazani MR. *Pac Sci*, 1994; **48(4)**:458-463.
17. Obadoni BO, Ochuko PO. *Global J Pure Appl Sci*, 2001; **8**:203-208.
18. Barakat MZ, Shehab SK, Darwish N, Zahermy EI. *Analyst Biochem*, 1973; **53**:225-245.
19. Van-Burden TP, Robinson EC. *J Agric Food Chem*, 1981; **1**:77-82.
20. Halliwell B, Gutteridge JMC. *FEBS Let*, 1981; **128(2)**:347-352.
21. Pulido R, Bravo L, Saura-Calixto F. *J Agric Food Chem*, 2000; **48(8)**:3396-3402.
22. Banerjee A, Dasgupta N, De B. *Food Chem*, 2005; 90:727-733.
23. Janario G, Linley MSY, Yap L, Liansolazaro L, Robles J. *e-J Sci Technol*, 2011; **2**:77-83.
24. Blois MS. *Nature*, 1958; **29**:1199-2000.
25. Decker EA, Barbara W. *J Agric Food Chem*, 1990; **38(3)**:674-677.
26. Khatiwora E, Adsul, VB, Kulkarni MM, Deshpande NR, Kashalkar RV. *Int J ChemTech Res*, 2010; **2(3)**:1698-1701.
27. Kano M, Takayanagi T, Harada, Makino K, Ishikawa K, Fumiyasu I. *J Biosci Biotechnol Biochem*, 2005; **69(5)**:979-988.
28. Prasad KN, Soundar D, Gyarahally RW, Mallikaruna AS. *J Sci Food Agri,* 2005; 85(9): 1461-1468.
29. Yadav N, Saxena J, Maheshwari M. *J Adv Sci Res*, 2021; **12(04)**: 227-232.
30. Daddala G., Rani A, Kumar A. *J Adv Sci Res*, 2022; **13(01)**:147-152.