International Journal of Pharmacognosy and Life Science

E-ISSN: 2707-2835
P-ISSN: 2707-2827
www.pharmacognosyjournal.com
IJPLS 2024; 5(2): 08-12
Received: 06-04-2024
Accepted: 11-05-2024

Dr. S Senthilkumar
Post Doctoral, Research Scholar, Manipur International University, Imphal, Manipur, India

Dr. C Kiruba Rani
Department of Biochemistry, Vellalar College for Women, Erode, Tamil Nadu, India

Corresponding Author:
Dr. S Senthilkumar
Post Doctoral, Research Scholar, Manipur International University, Imphal, Manipur, India

Antioxidant activities of ethanolic extract of *Acalypha indica* Linn

Dr. S Senthilkumar and Dr. C Kiruba Rani

DOI: https://doi.org/10.33545/27072827.2024.v5.i2a.120

Abstract
Oxidative stress, the excessive presence of reactive oxygen species (ROS), is suggested as a basal cause of aging as well as various degenerative and chronic diseases in human. Antioxidants are belived to play a very vital role in the body defence system against ROS. Plant - based antioxidants with their prominence have gained tremendous worldwide interest nowadays. *Acalypha indica* used various diseases medication which have potential source as natural antioxidants. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of single oxygen formation.

Keywords: Medicinal plant, antioxidant, free radicals, reactive oxygen species, oxidative stress

Introduction
Reactive oxygen species (ROS) is indispensable in many biological processes, mainly during cell differentiation, disease mechanism, immunity etc. [1]. Therefore, ROS is produced in normal metabolic reaction and is maintained at physiological levels by several endogenous antioxidant system. In addition, exogenous ROS may be generated from environmental pollutants, excessive alcohol consumption, radiation exposure, viral and bacterial infections, and others [2]. How ever, a condition in which ROS are excessively generated, oxidative stress is going be inevitable causing multiple cellular compartments, damage, cell injury or cell damage. The oxidative stress, triggered by the in balance between oxidants and antioxidants, eventually leads to many degenerative and chronic diseases in human [3, 4].
Medicinal plants play a vital role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly used plant based drugs or formulation to treat various human ailments because they contain the components of therapeutic value [5]. In addition, plant based drugs remain an source of therapeutic agents because of the ability, relatively cheaper cost and non-toxic nature when compared to modern medicine [6]. Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species.
ROS such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources [7]. Antioxidants from plant materials terminate the actin of free radicals there by protection the body from various diseases [8]. There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants.

Materials and Methods
Preparation of ethanolic extract
The extract and fraction were air-dried using the oven at 4:C until dried (Constant weight). The extract were dissolved and diluted using ethanol prior to assay (10mg dissolved up to a volume of 1 ml).

Ferric reducing / antioxidant power (FRAP) assay
FRAP (900 µl) reagent, prepared freshly and incubated at 37 °C, was mixed with 90 µl of distilled water and 30 µl of test sample. The test sample and reagent blank were incunated at

37 °C for 30 min. in a water bath. The final dilution of the test sample in the reaction mixture was 1:34. The FRAP reagent contained 2.5ml of 20 mmol / 1,2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40mmol / 1 HCL plus 205ml of 20mm0l/1 $Fe_3Cl.6H_2O$ and 25 ml of 0.8 mol. of acetate buffer ($_pH$ 3.6). at the end of incubation, the absorbance readings were taken immediately at 593 nm using a spectrophotometer. The values are expressed as mmol Fe (II) mg^{-1} extract [9].

DPPH radical scavenging assay

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The sample extract at various concentrations (100-500 µg) was taken and the volume was adjusted to 100 µl with methanol. 5ml of 0.1 mM methanol solution of DPPH was added and allowed to stand for 20 min. at 27 °C. The absorbance of the sample was measured at 517 nm and the percentage radical scavenging activity of the sample was calculated as follows.

% DPPH radical scavenging = (OD of control-OD of sample/ OD of control) × 100 activity.

The analysis was performed in triplicate. The sample concentrating providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration [10].
Tannic acid was used as a standard.

Hydroxyl radical scavenging assay

About 100-500 µg of solvent extract was added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%) and 1ml of Dimethyl Sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, $_pH$ 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90^0C for 15 min. in a water bath. After incubation, the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5% w/v). Three m;l of Nash reagent (75 g of ammonium acetate, 3ml of glacial acetic acid and 2ml of acetyl acetone were mixed and raised the volume to 1L with distilled water) was added and left at room temperature for 15 min. The intensity of the colour formed was measured at 412 nm against reagent blank [11].
The % hydroxyl radical scavenging activity was calculated as follows

% HRSA = (OD of control - OD of sample / OD of control × 100

Mannitol, a classical OH scavenger was used as a positive control

Superoxide radical scavenging assay

The assay was based on the capacity of the extract to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Each 3ml reaction mixture contained 50mM sodium phosphate buffer ($_pH$ 7.6), 20 mg riboflavin, 12M EDTA, 0.1 mg NBT and various concentrations (100 - 500 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590nm. The entire reaction assembly was enclosed in a box lined with aluminium foil [12]. The percentage inhibition of superoxide anoin generation was calculated as follows:

% inhibition = (OD of cotrol - OD of sample / OD of control × 100.

Quercetin was used as a positive control.

Nitric oxide radical scavenging assay

3ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline ($_pH$ 7.4) was mixed with different concentrations (100 - 500 µg / ml) of extract and incubated at room temperature for 15 min. After incubation time, 0.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was read at 546 nm [13].

% NO radical scavenging = (OD of control - OD of sample/ OD of control) $_X$100 activity. Curcumin was used as a standard.

Results and Discussion

FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenolics. The reducing capacity of a compound may serve as a significant indicator of potential antioxidant activity. However, the activities of antioxidants have been attributed to certain mechanisms namely chain initiation, decomposition of peroxides, reducing capacity and radical scavenging activity [15]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.
The present study showed a free radical scavenging activity of 186.05±2.9 mmol (Fe II)/mg extract by FRAP assay. Antioxidant activity is found to be linearly proportional to phenolic content of the plant. Oktay *et al.* [16]. Reported a strong positive relationship between total phenolic contents and antioxidant activity of plants. Similar observation was reported by Huda - Faujan *et al.* [17]. In methanolic extract of *Cosmos caudatus.*
From the present study, it is inferred that the phenolic compounds in the ethanolic extract of *A. indica* may act as an electron donor, thereby reducing the free radical generation. DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. DPPH assay is a simple and acceptable method to evaluate the antioxidative activity of compounds. DPPH is a stable free radical, which when encounters proton donors such as antioxidants, the radicals get quenched, absorbance gets reduced and thus used to measure the antioxidant activity of specific compound or plant extract [19, 20]. The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity of plant extract may be due to neutralization of DPPH radical either by transfer of hydrogen or of an electron [21].
The results of the present study revealed an effective free radical scavenging activity of ethanolic extract of *A. Indica* in the DPPH assay as shown in Table 1. This is in correlation with an earlier report of the Molan *et al.* [22]. Revealed that the essential oil of *Pterodon emarginatus*

seeds containing phenol showed DPPH scavenging activity with IC_{50} value of 163.22 and the phenolic compound may reduce the oxidative stress which causes damage to carbohydrates, lipids, proteins and nucleic acids [24]. These evidences support the present study that the phenolic compounds of ethanolic extract of *A. indica*, make a significant contribution on the antioxidant activity in scavenging the DPPH radical.

Hydroxyl radical is one of the potent reactive oxygen species in the biological system [25]. The hydroxyl radical in the cells can easily cross cell membranes and react with most of the biomolecules and cause tissue damage and cell death. Hence, removal of hydroxyl radical is very important for the protection of living system [26].

In the present study, the results of hydroxyl radical scavenging activity of ethanolic extract of *A. indica* exhibited in Table 2 which inferred that the ethanolic extract of *A. indica* may be considered as a good scavenger of hydroxyl radicals. If any plant extract of drug scavengers the hydroxyl radical, they may either scavenge the radical or may chelate the Fe^{2+} ion, making them unbelievable for the Fenton's reaction. Plant extracts containing polyphenolics are reported to quench oxygen derived free radicals by donating a hydrogen atom or an electron to the free radicals or neutralizing the free radicals by their chelating ability

The results obtained in the present study is in consonance with the previous reports of Hazra *et al.* [27]. In *Spondias pinnata* methanol extract. The present investigation also revealed that the ethanolic extract of *A. indica* is shown to be significant source of natural antioxidants, which are responsible for the hydroxyl radical scavenging activity.

Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell damaging free radicals and oxidizing agents. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen. Superoxide anion radical and hydrogen peroxide radical together react to form singlet oxygen and OH radical, which are the most reactive oxygen species among all the ROS. It has the ability to react with several biological materials by hydrogen withdrawal, double bond addition, electron transfer and radical formation and initiates auto-oxidation, polymerization and fragmentation. Hydroxyl radical can cause sugar fragmentation. Base loss and leakage of DNA strands furthermore, superoxide anion radical and its derivatives can cause damage in lipids, proteins and DNA. Hence, it is of great importance to scavenge superoxide anion radical [28].

The results presented in table 3 showed an increase of percentage scavenging activity, which indicated the consumption of superoxide anion in the reaction mixture by ethanolic extract of *A. indica.* Superoxide scavenging ability of plant extract migt be primarily due to the presence of flavonoids [29]. Flavonoids are effective antioxidants mainly because they scavenge superoxide anions. Based on the above results of the percentage scavenging capacity and IC_{50} values, it was found that the ethanolic extract of *A. indica* is more effective in scavenging superoxide radicals that might be due to the presence of flavonoids. This result correlates with an earlier study of Olayinka *et al.* [30]. In *Helichrysum longifolium* aqueous extract.

Nitric oxide, a gaseous free radical and is relatively less reactive. But its metabolic product peroxynitrite, formed after reacting with oxygen is extremely reactive an directly induce toxic reactions such as SH group oxidation, protein - tyrosine nitration, lipid peroxidation and DNA modification. Nitric oxide has been demonstrated to participate in the beta cell damage during STZ - induced diabetes. Nitric oxide plays an important role in various inflammatory processes. Sustained level of production of this radical is directly toxic to tissues and contribute to vascular collapse associated with septic shock, whereas chronic exposure of nitric oxide radical is associated with various carcinoimas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis. The toxicity of NO increases greatly when it reacts with superoxide radical, producing the highly reactive peroxynitrote ($ONOO^-$) anion [31].

The results presented in table 4 expressed a potent nitrogen scavenging scavenging activity of ethanolic extract of *A. indica* obtained in the present study proved that *A. indica* has significant antioxidant and free radical scavenging activity, which might be attributed to its flavonoids, phenolic contents and other phytochemical constituents. Hence, the selected plant extract may be used as therapeutic agent in preventing oxidative stress related degenerative diseases.

Table 1: DPPH radical scavenging activity of ethanolic extract of *A. indica*

Sample	Concentration (µg/ml)	Percentage activity	IC_{50}
Ethanolic extract of *A. indica*	100	10.04±0.729	476.19
	200	21.04±0.317	
	300	33.10±0.449	
	400	41.88±1.421	
	500	52.37±0.400	
Tannic acid	2	16.09±2.6	13.1
	4	28.84±0.7	
	6	33.26±1.2	
	8	36.87±0.7	
	10	41.61±1.67	

Values are mean of triplicate determination; ± standard deviation

Table 2: Hydroxyl radical scavenging activity of ethanolic extract of *A. indica*

Sample	Concentration (µg/ml)	Percentage activity	IC_{50}
Ethanolic extract of *A. indica*	100	8.92±0.308	574.71
	200	17.45± 0.178	
	300	28.27± 0.122	
	400	35.74± 0.216	
	500	42.16± 0.394	
Mannitol	10	16.03±0.1	38.3
	20	18.11±1.1	
	30	33.11±1.21	
	40	55.12±1.1	
	50	67.55±1.2	

Values are mean of triplicate determination; ± = Standard deviation

Table 3: Superoxide radical scavenging activity of ethanolic extract of *A. indica*

Sample	Concentration (µg/ml)	Percentage activity	IC_{50}
Ethanolic extract of *A. indica*	100	8.96±0.394	568.18
	200	19.27±0.333	
	300	28.26±0.658	
	400	35.66±0.441	
	500	42.62±0.500	
Quercetin	10	9.1±0.1	52.1
	20	18.2±0.11	
	30	22.1±0.21	
	40	35.2±0.13	
	50	48.3±0.14	

Values are mean of triplicate determination; ± = Standard deviation

Table 4: Nitric oxide radical scavenging activity of ethanolic extract of *A. indica*

Sample	Concentration (µg/ml)	Percentage activity	IC_{50}
Ethanolic extract of *A. indica*	100	11.40±0.147	427.35
	200	23.98±0.564	
	300	36.63±0.250	
	400	47.67±0.306	
	500	56.87±0.278	
Curcumin	10	15.1±01	43.1
	20	25.2±0.2	
	30	30.3±1.1	
	40	45.4±1.21	
	50	60.4±1.3	

Values are mean of triplicate determination; ± = Standard deviation

Conclusion

Based on the findings presented, it is evident that Acalypha indica possesses significant antioxidant properties, as demonstrated by its effective scavenging activities against various free radicals such as DPPH, hydroxyl radicals, superoxide anions, and nitric oxide radicals. These activities are crucial in combating oxidative stress, which is implicated in the pathogenesis of numerous degenerative diseases. The presence of phenolic compounds in the ethanolic extract of A. indica appears to play a pivotal role in its antioxidant capabilities, acting as electron donors and neutralizing free radicals.

The results from this study underscore the potential of A. indica as a valuable source of natural antioxidants. Such properties are particularly promising amidst the growing interest in plant-based therapies due to their perceived safety, cost-effectiveness, and ability to mitigate oxidative damage in biological systems. Further research into the specific bioactive compounds responsible for these antioxidant activities could pave the way for the development of therapeutic interventions targeting oxidative stress-related diseases. This study contributes to the expanding body of knowledge on medicinal plants and their role in promoting human health and well-being.

References

1. Baunthiyal M, Singh V, Dwivedi S. Insights of antioxidants as molecule for drug discovery. Int J Pharmacol. 2017;13(7):874-889.
2. Caccia Puoti F. Oxidative stress as mother of many human diseases at strong clinical impact. J Cardiovasc Med. Cardiol. 2016;3(1):01-06.
3. Gangwar M, Gautam MK, Sharma AK, Tripathi YB, Goel RK, Nath G, *et al*. Antioxidant capacity and radical scavenging effect of polyphenol rich *Mallotus philippensis* fruit extract on human erythrocyte: An *in vitro* study. Sci World J. 2014;2014:1-12.
4. Matseehke V, Theiss C, Matschke J. Oxidative stress: The lowest common denominator of multiple diseases. Neural Regen Res. 2019;14(2):238-241.
5. Agbor AG, Ngogang YJ. Toxicity of herbal preparations. Cam J Ethnobot. 2005;1:23-28.
6. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 1990;186:1-85.

7. Roja G, Rao PS. Anticancer compounds from tissue cultures of medicinal plants. J Herbs Spices Med Plants. 2000;7:71-102.
8. Hussain SR, Cillard J, Collard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry. 1987;26:2489-2491.
9. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem. 2000;48:3396-3402.
10. Blois MS. Antioxidant determination by the use of a stable free radical. Nature. 1958;181:1199-1200.
11. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry. 1991;20:6006-6012.
12. Beauchamp C, Fridovich I. Superoxide: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44:276-287.
13. Sreejayan N, Rao M. Nitric oxide radical scavenging assay. J Biochem. 1997;6:1095-1097.
14. Luximon-Ramma A, Bahorun T, Soobrattee AM, Aruoma OI. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Acacia fistula. J Agric Food Chem. 2002;50:5042-5047.
15. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr. Biochem. 1996;7:66-76.
16. Oktay M, Gülçin I, Küfrevioğlu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Food Sci. Technol. Int. 2003;36:263-271.
17. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidant activity of methanolic extract of *Cosmos caudatus* containing phenolic compounds. Afr. J Biotechnol. 2009;8(3):484-489.
18. Chen CW, Ho CT. Antioxidant properties of polyphenols extracted from green and black teas. J Food Lipids. 1995;2:35-46.
19. Koleva IT, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal. 2002;13:8-17.
20. Qureshi MN, Kuchekar BS, Logade NA, Haleem NH. *In vitro* antioxidant and *in vivo* hepatoprotective activity of *Leucas ciliata* leaves. Rec Nat Prod. 2010;4(2):124-130.
21. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric. Food Chem. 1992;40:945-948.
22. Molan AL, Faraj AM, Mahady AS. Antioxidant activity and phenolic content of some medicinal plants traditionally used in northern Iraq. Phytopharmacology. 2012;2(2):224-233.
23. Dutra RC, Leite MN, Barbosa NR. Quantification of phenolic constituents and antioxidant activity of *Pterodon emarginatus* vogel seeds. Int. J Mol. Sci. 2008;9:606-614.
24. Pryor WA, Houk KN, Foote CS. Free radicals biology and medicine. Am J Physiol. Regul. Integr. Comp Physiol. 2006;291(3):491-511.
25. Halliwell B, Gutteridge JM. Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS Lett. 1981;128:347-352.
26. Yang JX, Guo J, Yuan JF. *In vitro* antioxidant properties of rutin. Food Sci Technol. 2008;41:1060-1066.
27. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complement Altern Med. 2008;8:63.
28. Sathish M, Tharani CS, Niraimathi V. Free radical scavenging activity of various extracts from roots of *Clerodendrum phlomidis* (L). Res. J Pharm Biol. Chem. Sci. 2011;2:752-761.
29. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric. Food Chem. 2001;49(11):5165-5170.
30. Olayinka A, Aiyegoro O, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med. 2010;10:21.
31. Huie RE, Padmaja S. The reaction of NO with superoxide. Free Radic. Res. Commun. 1993;18:195-199.
32. Balakrishnan N, Panda AB, Raj NR, Shrivastava A, Prathani R. The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn. root. Asian J Res. Chem. 2009;2(2):148-150.