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EVALUATION OF IN VITRO ANTIOXIDANT PROPERTIES OF ETHANOLIC AND AQUEOUS EXTRACTS OF ACHYRANTHES ASPERA L.

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ABSTRACT

Achyranthes aspera (Amaranthaceae) is an important medicinal herb found as a weed and its parts are used in traditional systems of medicine. Plant and plant products are being used as a source of medicine since long. Plant extracts increasingly used as phytotherapeutics and are still a large source of natural antioxidant. Antioxidant activity was assessed by using total antioxidant activity, DPPH scavenging activity, nitric oxide scavenging activity, SOD scavenging activity using appropriate assay systems compared to natural and synthetic antioxidants. Ascorbic acid was kept as standard. IC_{50} values were also observed. Thus, it can be concluded that the whole plant of *Achyranthes aspera* can be used as an accessible source of natural antioxidants with consequent health benefits.

KEYWORDS: *Achyranthes aspera*, *in vitro* antioxidant activity, DPPH radical scavenging assay, total antioxidant activity, nitric oxide scavenging activity, superoxide dismutase scavenging activity.

INTRODUCTION

Plants have been used as medicines for thousands of years. People depend on plants for several purposes like for wood, timber, non-timber forest products, food, medicine etc. (Jain *et al.*, 2005). They have always been used as a rich source of biologically active drugs and have numerous traditional uses to serve mankind for many thousand years (Kirtikar *et al.*, 1999). Now a day, they are used widely because of growing awareness of people towards

unwanted side effects and high cost of the allopathic medicines which makes them beyond the reach of common people.

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Achyranthes aspera* which is commonly known as Latjeera (Hindi) & Rough Chaff tree (English).

Achyranthes aspera L. (Latjeera) is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour, branches terete or absolutely quadrangular, striate, pubescent, leaves thick, 3.8 - 6.3 × 22.5 - 4.5 cm, ovate – elliptic or obovate rounded, finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long, flowers greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown (Jain *et al.*, 2006).

Oxidative Stress

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation in vivo and in vitro and create oxidative stress which results in numerous diseases and disorders (Halliwell, 1994; Rackova *et al*., 2007).

Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals and hydroxyl radicals (OH.), as well as non-free radicals and singlet oxygen (Halliwell, 1994). In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc (Buyukokuroglu *et al*., 2001).

When oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack on lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko *et al*., 2006).

Natural sources of antioxidants

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006).

***In vitro* Methods**

Various methods are used to investigate the antioxidant property of sample (diets, plants extracts, commercial antioxidant etc.). The objective of this review article is to accumulate all probable methods that are used to evaluate the antoxidant property of various samples. Two review articles have been published (Chanda and Dave, 2009 and Badarinath *et al*., 2010) on *in vitro* evaluation of antioxidant activity. In this article, attempts have been taken to include in vivo too and to analyze the frequency of the use of different methods.

The present study was to evaluate the antioxidant activity of aqueous and ethanolic extract of *achyranthes aspera* L. *in vitro* models.

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials

The plant species namely *Achyanthes aspera* L. plant was collected by in and around Koothanallur, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997). The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an

electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the aqueous extract

The plant material (Whole plant) was shade dried and coarsely powdered with electrical blender. 200g of *Achyranthes aspera* was mixed with 1200ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extracts was prepared according to the methodology of |Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40^0C – 50^0C). The paste form of the extracts was put in an air tight container stored in refrigerator.

***In vitro* antioxidant activity**

Total antioxidant capacity

To determine the total antioxidant capacity was performed by the method of Sirwaikar Annie *et al*. (2004). To 1 ml of extract of different concentration was treated with 1 ml of reagent solution (0.6mM sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in eppendorf tube. Capped tubes were incubated in thermal block at 95^oC for 90min. after cooling to room temperature; the absorbance was measured at 695nm against blank. The activity was compared with ascorbic acid standard.

% of scavenging total antioxidant activity = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

DPPH scavenging Activity

The DPPH Scavenging Activity was determined by the method of Gulcin *et al*. (2004). The solution of DPPH was prepared by adding 4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 µl methanol; it was protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solutions were added to 3ml methanol and

absorbance was taken immediately at 517nm for control reading. Each of the samples of various concentrations was then further diluted with methanol up to 3µl and to each 150 µl DPPH was added. Absorbance was taken after 15 min. at 517nm using methanol as blank on UV-visible spectrometer. The IC50 values for each drug compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

Nitric Oxide scavenging activity

The Nitric Oxide Scavenging Activity was determined by the method of Kamble *et al*. (2011). Sodium nitroprusside (SNP-5mM) in phosphate-buffer saline was mixed with an equivalent amount of methanol to get the control. Methanol served as blank. Methanol was added to test solutions at different concentrations to make up a volume of 3ml and incubated at room temperature (270C) for 90 minutes. This incubated solution (1.5 ml) was added to 1.5 ml of Greiss Reagent. Absorbance at 546 nm was noted using UV–VIS spectrophotometer.

$$\% \text{ of Nitric oxide Scavenging activity} = \{(\text{Control Abs.—Sample Abs.})/\text{Control Abs}\} \times 100$$

Superoxide radical scavenging activity

The Superoxide radical Scavenging Activity was determined by the method of Nishimiki *et al.* (2004). About 1 ml of nitro blue tetrazolium, 1ml NADH in 100mM phosphate buffer at pH 7.8 and 0.1 ml of sample solutions of different concentrations were mixed. The reaction was started by adding 100µl PMS and the reaction mixture was incubated at 25°C for 5 minutes. The absorbance of the mixture was measured at 560nm.

$$\% \text{ of Superoxide radical scavenging activity} = \{(\text{Control Abs. - Sample Abs.})/\text{Control Abs}\} \times 100$$

RESULTS AND DISCUSSION

Antioxidant activity

Plants are important source of potential compounds for the development of new therapeutic agents. *In vitro* antioxidant activity of *Achyranthes aspera* was investigated in the present study by total antioxidant activity, DPPH scavenging activity, nitric oxide scavenging

activity, superoxide dismutase radical scavenging activity. It is probably due to the presence of respective phytochemicals like flavonoids and phenolics etc. in these species (Akter *et al*., 2010).

Total antioxidant activity

Total antioxidant activity of aqueous and ethanolic extract of *Achyranthes aspera* and ascorbic acid values were presented in Table 1 and Figure 1. The half maximal inhibitory concentration (IC_{50}) of aqueous and ethanolic extract was found to be 103.51µg/ml and 99.60µg/ml respectively.

The total antioxidant activity of ethanolic extract of *Achyranthes aspera* was estimated from their ability and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts (Prieto *et al*., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay *et al*., 2003). The statement has been justified in the current study where the aqueous extract of *Achyranthes aspera* showed maximum antioxidant activity.

DPPH radical scavenging activity

The DPPH radical scavenging activity of aqueous and ethanolic extract of *Achyranthes aspera* and ascorbic acid values were presented in the Table 2 and Figure 2. The half maximal inhibitory concentration (IC_{50}) of aqueous and ethanolic extract was found to be 71.12µg/ml and 86.80µg/ml respectively. The ethanolic extract has potent DPPH radical scavenging activity more than aqueous extract.

The percentage inhibitions at various concentrations (20, 40, 60, 80 and 100 µg /ml) of aqueous and ethanolic extract of *Achyranthes aspera* was calculated and plotted. The values were estimated from the percentageof inhibition various concentration (Rahman *et al*., 2013).

Aqueous and ethanolic extract of *Achyranthes aspera* showed the significant results were observed. The activity is due to the presence of flavonoid compounds present in methanol extract. Thus choosing the appropriate solvent is one of the most important factors for obtaining extracts with a high content of bioactive compounds and antioxidant activity (Park *et al*., 2008).

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity of aqueous and ethanolic extract of *Achyranthes aspera* and ascorbic acid values were presented in the Table 3 and Figure 3. Nitric oxide is a very unstable species and reacting with oxygen molecule producing stable nitrate and nitrite which can be estimated by using griess reagent. The half maximal inhibitory concentration (IC_{50}) of aqueous and ethanolic extract was found to be 93.98µg/ml and 95.93µg/ml respectively. The reductive capability was found to increase with increasing concentration in ethanolic extract.

Nitric oxide is a potent pleiotrophic inhibition of physiological processes such as smooth muscle relxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radical that ply many roles s an effectors molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumour activities (Hagerman, 1998).

The increased nitric oxide radical scavenging activity was observed in ethanolic extract of tested plant of these plant *Achyranthes aspera* showed better scavenging capacity. The nitric oxide radical scavenging potentiality may be due to antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibit the generation of nitrites.

Superoxide dismutase radical scavenging activity

Superoxide anion radical is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Superoxide anion, which is reduced form of molecular oxygen, has been implicated in the containing oxidation reactions associated with aging (Cotelle *et al*., 1996).

Superoxide dismutase radical scavenging activity of aqueous and ethanolic extract of *Achyranthes aspera* and ascorbic acid values were presented in the Table 4 and Figure 4. The half maximal inhibitory concentration (IC_{50}) of aqueous and ethanolic extract was found to be 61.50µg/ml and 60.45µg/ml respectively. The aqueous extract has potent nitric oxide radical scavenging activity more than ethanolic extract.

Table 1: Total Antioxidant scavenging activity of *Achyranthes aspera.*

S. No.	Concentration (µg/ml)	Aqueous extract		Ethanolic extract	
		% of Inhibition	IC_{50} Value (µg/ml)	% of Inhibition	IC_{50} Value (µg/ml)
1	20	20.06±0.6	103.51	23.8±2.7	99.60
2	40	23.4±0.85		25.4±2.5	
3	60	27.6±0.9		28.6±1.1	
4	80	34.4±1.06		32.3±0.9	
5	100	48.3±2.1		50.2±0.2	
6	Ascorbic acid (standard)	40.1±0.85	124.68	42.5±2.5	117.64

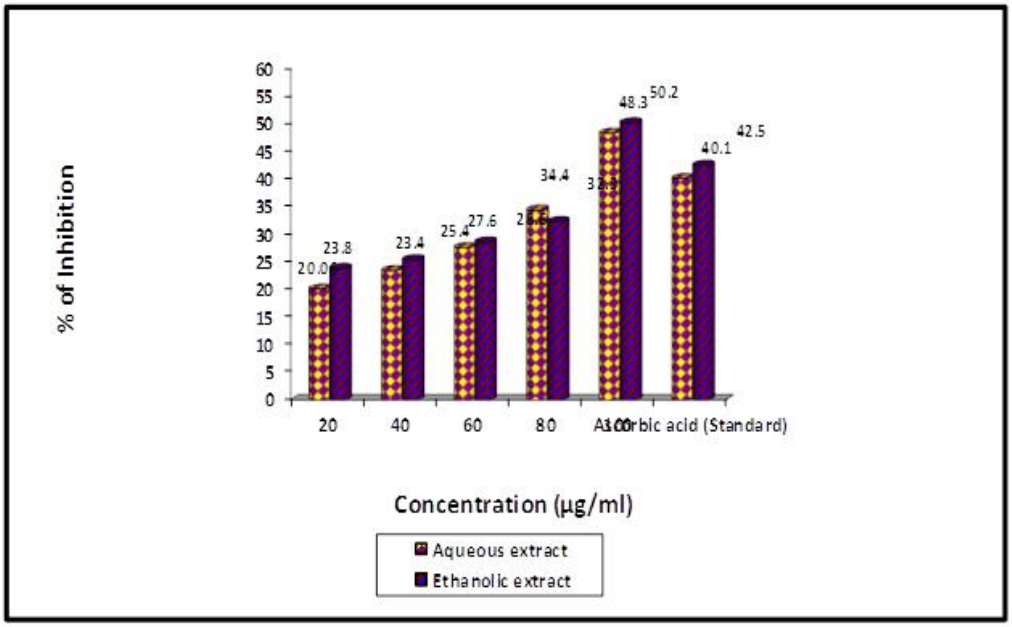

Figure 1: Total Antioxidant scavenging activity of *Achyranthes aspera.*

Table 2: DPPH scavenging activity of *Achyranthes aspera.*

S. No.	Concentration (µg/ml)	Aqueous extract		Ethanolic extract	
		% of Inhibition	IC_{50} Value (µg/ml)	% of Inhibition	IC_{50} Value (µg/ml)
1.	20	34.7±1.5	71.12	26.06±1.5	86.80
2.	40	42.5±0.6		34.3±2.05	
3.	60	48.1±0.9		43.1±1.7	
4.	80	54.2±1.3		51.8±1.2	
5.	100	70.3±0.5		57.6±1.02	
6.	Ascorbic acid (standard)	47.2±2.5	105.93	45.2±1.3	110.61

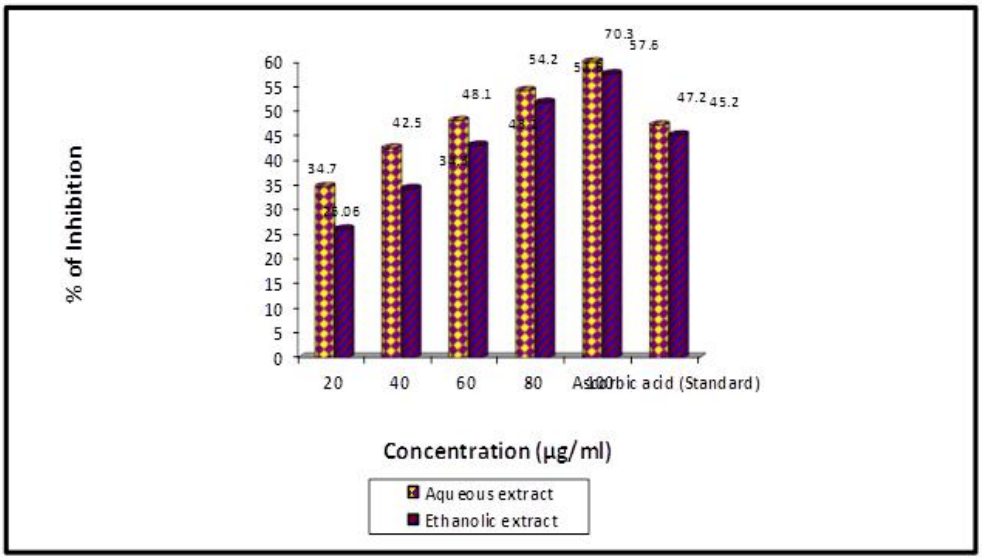

Figure 2: DPPH scavenging activity of *Achyranthes aspera.*

Table 3: Nitric oxide scavenging activity of *Achyranthes aspera*.

S. No.	Concentration (µg/ml)	Aqueous extract		Ethanolic extract	
		% of Inhibition	IC_{50} Value (µg/ml)	% of Inhibition	IC_{50} Value (µg/ml)
1.	20	47.9±2.05	93.98	49.4±0.8	95.23
2.	40	58.3±2.02		60.3±1.7	
3.	60	67.6±1.52		68.6±1.4	
4.	80	74.4±1.9		75.7±1.1	
5.	100	81.3±1.1		82.7±1.2	
6.	Ascorbic acid (standard)	56.2±0.5	133.68	58.8±2.1	154.32

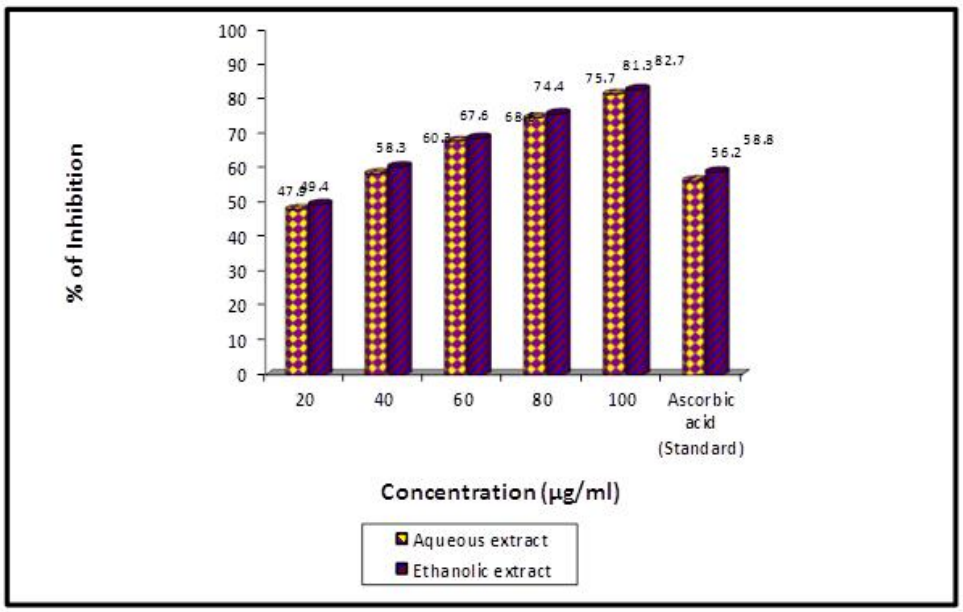

Figure 3: Nitric oxide scavenging activity of *Achyranthes aspera.*

Table 4: Superoxide dismutase scavenging activity of *Achyranthes aspera*.

S. No.	Concentration (µg/ml)	Aqueous extract		Ethanolic extract	
		% of Inhibition	IC_{50} Value (µg/ml)	% of Inhibition	IC_{50} Value (µg/ml)
1	20	23.3±0.8	61.50	22±0.4	60.45
2	40	32.1±1.9		31.4±3.0	
3	60	37.9±0.5		37.5±1.0	
4	80	43.6±1.1		43.3±1.2	
5	100	53.2±0.9		52.5±2.1	
6	Ascorbic acid (standard)	37.4±0.1	88.96	32.4±1.2	85.03

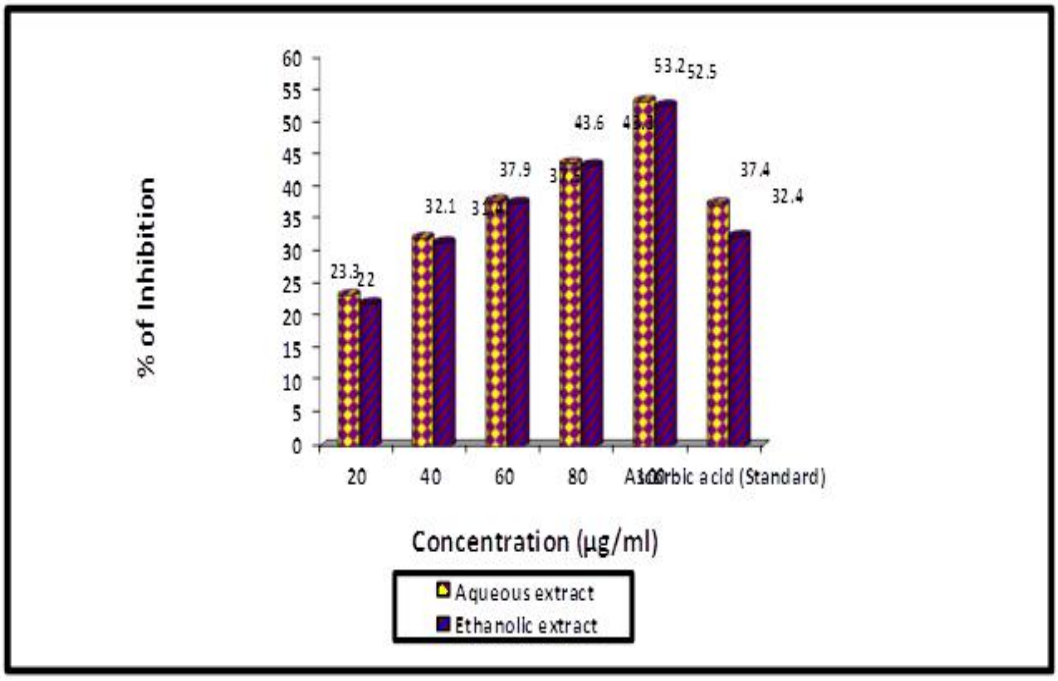

Figure 4: Superoxide dismutase scavenging activity of *Achyranthes aspera*.

CONCLUSION

In the present study, free radical scavenging potential of aqueous and ethanolic extracts of the whole plant of *Achyranthes aspera* was assessed by measuring its capability for total antioxidant activity, DPPH scavenging activity, nitric oxide scavenging activity, SOD scavenging activity using appropriate assay systems compared to natural and synthetic antioxidants. It showed different level of antioxidant activity. Our study demonstrated that the extracts of *Achyranthes aspera* are a potential source of antioxidants and thus could prevent many radical related diseases. However, further detailed studies are required to determine the active components responsible for these effects and mechanism pathway.

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