International Journal of Phytomedicine 4 (2012) 243-247
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Eradicating potential of *Achyranthes aspera*

Vivek Dave[1*], Swapnil Sharma[1], Km. Ruby[1], Sachdev Yadav[1], Jitendra Bajaj[2]

*Corresponding author:

Vivek Dave

[1]Department of Pharmacy, Banasthali University, Banasthali-304022
[2]Department of Pharmacy, NRI Institute of Pharmacy, Bhopal, M.P 462021

Abstract

The antioxidant activity of *Achyranthes aspera* methanolic aerial part extracts was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power assays. Quantitative estimation of total flavonoid and phenolic content was performed followed by thin layer chromatography (TLC) to confirm the presence of secondary metabolites in the extract.

From results of DPPH and ferric-reducing antioxidant power assays, it was found that methanolic aerial part extracts of *Achyranthes aspera* precipitates strong antioxidant ($P < 0.005$) activity compared to the ascorbic acid. The concentrations of total flavonoid and total phenolic content were found to be 47.6 mg RuE/g and 28.66 mg GAE/g respectively. The TLC study showed distinguished spots and revealed the presence secondary metabolites.

Keywords: *Achyranthes aspera,* methenolic extract, antioxidant activity, quantitative analysis

Introduction

Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves.[1] Many human diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of pro-oxidants.[2] Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease.[3-4] Plants are potential sources of natural antioxidants.[5] The extracts of medicinal plants and natural products have become a great source of antioxidant and anti-ageing properties.[6]

The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods.[7-8] Principal sources of antioxidant include herbs, spices, and medicinal plants. Natural antioxidants from dietary plants are reported to prevent oxidative damage by free radical and active oxygen, and they also prevent the occurrence of disease, aging, and cancer.[9]

Achyranthes aspera L. belong to Family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India. [10-11] It is known as "Prickly chaff flower" in English and Chirchita, Onga , Latjeera, Apamarga in local language. The plant is highly esteemed by traditional healers and researcher used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases etc.[12-16]

Due to the increasing interest in the relationship between antioxidants and diseases, there is a need to get an overall measure of the antioxidant activity of extracts from *Achyranthes aspera* plant. In this study, we evaluated the possible antioxidant effects of methanolic extract from the aerial part in vitro antioxidant tests including total phenolic determination, flavonoids determination, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, reducing power.

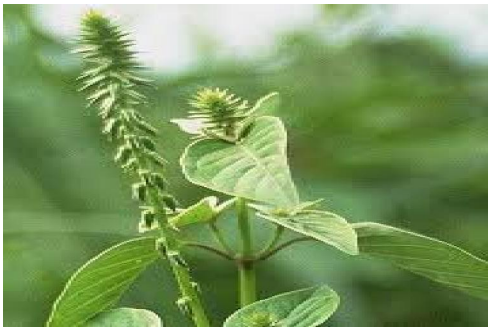

Fig: 1. Plants of Achyranthes *aspera*

Materials and methods

Plant material

The aerial part of *Achyranthes aspera* was collected from Bhopal, M.P, India. The specimen was identified by Dr.Zeaul hasan (Assistant professor) Department of Botany, Safia college Bhopal, M.P, India. A voucher specimen (257/Bot/saifia/11) has been deposited for future reference in the herbarium, department of botany, Safia College, Bhopal, M.P, India.

Preparation of extract

The aerial part of *Achyranthes aspera* plant were shade dried and cursed to make coarse powder. The plant powder (300gm) was defatted with petroleum ether (40^0C- 60^0C) in a soxhlet apparatus. The defatted marc macerated with methanol using soxhlet until exhaustion to obtained methanolic extract. The residue was concentrated under rotary evaporator and finally dried and store in dessicator. The yield of the dried methanolic extract was found to be 8% of the dried plant material.

Total Flavonoid Determination

Total flavonoid content of methanolic extract of *Achyranthes aspera* was evaluated according to the method Chang *et al.*, (2002) with minor modification using rutin as a standard. Briefly, extract or standard solutions (0.25 ml) were mixed with 1.25 ml distilled water and 75 µl 5% $NaNO_2$. After 6 min, 75 µl of 10% $AlCl_3$ was added. After another 5 min, 0.5 ml of 1 M NaOH was added to the mixture. Immediately, the absorbance of the mixture was determined at 510 nm versus prepared water blank. Total flavonoids content was expressed as µg/mg rutin equivalents (RE)[17].

Total Phenolic determination

Total phenolic content of methanolic extract of *Achyranthes aspera* was evaluated according to the method McDonald *et al.*, (2001). 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) was mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate is added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. Gallic acid was used as positive controls. The total phenolic content is expressed in terms of standard equivalent (µg/mg of extracted compound)[18].

1, 1-Dipheny-2-Picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of methanolic extract of *Achyranthes aspera* and L-ascorbic acid (Vitamin C) was measured according to the method Koleva *et al.*, (2002) in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. For assessment of DPPH radical scavenging activity DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid (10-60 µg/ml) and extract (1-10µg/ml). After that 5ml of sample solution was mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517 nm. IC_{50} values denoted the concentration of sample, which is required to scavenge 50% of DPPH free radicals[19].

Determination of Reducing Power

The reducing power of extract was determined by the method of Yen and Duh (1993). Different concentrations of extract and reference (Ascorbic acid) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated for 20 min at 50 °C. After incubation, 2.5 ml of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation at 650×g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm[20].

Statistical analyses

The experiments were done in triplicate. Data were expressed as mean ± S.D. The mean of groups was compared by one- way ANOVA test and comparison of means was done by post hoc of Tukey. The differences were considered significant when $p < 0.05$ and $p<0.001$.

Thin Layer Chromatography

Thin layer chromatography was done according to the method Wagner H *et al.*, (1984) [21] Methanolic extract of *Achyranthes aspera* were subjected to thin layer chromatographic studies, to find out the probable number of secondary metabolites present in this and presented secondary metabolites identified by R_f value which was calculated as follows.

$$R_f = \frac{\text{Distance traveled by the sample}}{\text{Distance traveled by the solvent}}$$

Results and discussion

Total flavonoid determination

Hydroxyl radical have high affinity with aromatic compounds like flavonoid. The antioxidant power of flavonoid depends upon the number and configuration of phenolic hydroxyl groups in the molecules and also upon glycosylation and configuration of other constituent. Flavonoids are potent antioxidants having characteristics of scavenging free radical, chelating metal and inhibiting lipid peroxidation. The total flavonoid in the methanolic extracts of *Achyranthes Aspera* was found to be 47.6 mg RuE/g (Table2.) Rutin was used as a standard (Table 1).

Table: 1. Total flavonoid Content of Rutin

Sr.No.	conc.(µg/ml)	Absorbance (at 510 nm)
1	10	0.005± 0.130
2	20	0.008± 0.129
3	40	0.011± 0.127
4	60	0.017± 0.131
5	80	0.02± 0.123
6	100	0.026± 0.133

Values are expressed as mean ± SEM of 3 observations

Table: 2 Total flavonoid content of methanolic extract of *Achyranthes aspera*

S. No.	Conc	abs.	Rutin equivalent
1	1 µg/ml	0.098	47.6 mg

Table No: 3. Quantitative estimation of Gallic acid

Sr.No.	conc.(µg/ml)	Absorbance (at 760 nm)
1	1	0.05± 0.121
2	5	0.053± 0.111
3	10	0.061± 0.113
4	50	0.132± 0.126
5	100	0.283± 0.123
6	150	0.4± 0.128

Values are expressed as mean ± SEM of 3 observations

Table 4: Total phenolic content of methanolic extract of *Achyranthes aspera*

S.no.	Conc	abs.	Gallic acid Phenolic content
1	1 mg/ml	0.725	28.66 mg

1, 1-Dipheny-2-Picrylhydrazyl (DPPH) radical scavenging activity

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and HIV.[23] Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts.[19] The free radical scavenging capacity of ascorbic acid(Table 5.) and methanolic extract of *Achyranthes Aspera* (Table 6) was tested by its ability to bleach the stable DDPH radical. This assay provided information on the reactivity of the test compound with a stable free radical since its odd electron DPPH gives strong absorption bands at 517nm in visible spectroscopy (deep violet color).The concentration needed for 50% inhibition of methanolic extract and L-ascorbic acid was found to be 11.10 µg/ml, and 31.802 µg/ml respectively (Table 7.).

Table: 6. IC_{50} Determination of Extract

Sr.No.	Conc (µg/ml)	% Inhibition of DPPH
1	10	25.95± 0.013
2	20	35.88± 0.110
3	30	47.33± 0.124
4	40	60.31± 0.128
5	50	72.52± 0.019
6	60	79.39± 0.016

Values are expressed as mean ± SEM of 3 observations

Table: 7. IC_{50} value of ascorbic acid and *Achyranthes aspera* methanolic extract

Sr. No.	Sample	IC_{50}
1	Ascorbic acid	11.10 µg/ml
2	*Achyranthes aspera* methanolic extract	31.802 µg/ml

Reducing power (RP)

Table 8 and Table 9 showed the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of methanolic extract of *Achyranthes aspera* was very potent and the power of the extract was increased with quality of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of ascorbic acid. Increased absorbance of the reaction indicated increased reducing power

Table: 8. Determination of reducing power of ascorbic acid

Sr. No.	Conc(µg/ml)	Absorbance (at 700 nm)
1	10	0.428 ± 0.0202
2	20	0.607 ± 0.0081
3	30	0.697 ± 0.0066
4	40	0.736 ± 0.0054
5	50	0.747 ± 0.0156
6	60	0.790 ± 0.0103

Values are expressed as mean ± SEM of 3 observations

Table: 9. Determination of reducing power of extract

Sr. No.	Conc(µg/ml)	Absorbance (at 700 nm)
1	10	0.343± 0.0102
2	20	0.463± 0.072
3	30	0.495± 0.072
4	40	0.673± 0.092
5	50	0.679± 0.093
6	60	0.783± 0.0107

Values are expressed as mean ± SEM of 3 observations

Thin layer chromatography

The identification of major phytoconsitutents in the methanolic extract of *Achyranthes aspera* by the use of TLC was also studied to further confirm the presence of the possible secondary metabolites and this was represent in the Fig.6. The solvent system, number of spot and R_f values of extract show in Table 10.

Table: 10 Thin layer chromatography

Test extract	Solvent system	Number of Spots	R_f values
Methanol	Butanol: Glacial Acetic Acid: Water (8:2:10) Hexane : EA : GAA (10:5:0.1) Toluene : EA : Formic Acid (6.7:0.75:0.15)	2 2 6	0.846, 0.961 0.884, 0.953 0.106, 0.262, 0.356, 0.425, 0.652, 0.831

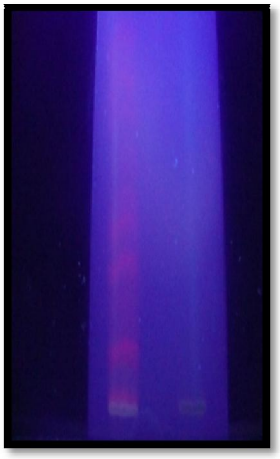

Fig. 6: TLC of Methanolic extract of Achyranthes *aspera*

(toluene: ethylacetate: formic acid) R_f value : 0.106, 0.262, 0.356, 0.425, 0.652, 0.831

Plant phenolics are a major group of secondary metabolites acting as primary antioxidants. Therefore, it was reasonable to determine the total phenolic content in the plant extract. The result shows that the phenolic content of methanolic extract of *Achyranthes aspera* is 28.66 mg GAE/g and the radical scavenging activity is 31.80µg/ml likely to be due to the phenolics, however, phenols may not be solely responsible for the antioxidant activity. In general, extracts with high antioxidant activity show a high phenolic content. Plant extracts with high phenolic contents also show high flavonoid content which is 47.6 mg RuE/g The reducing power increased with the increasing concentration of sample. Methanol extract of plant showed significant activities when compared to the ascorbic acid which was used as a control. Thin layer chromatography was also performed to confirm the presence of secondary metabolites in the extract using the solvent system toluene: ethyl acetate:

formic acid (6.7:0.75:0.15) showed the five spots of secondary metabolites.

Conclusion

Results of this analysis suggest that the species *Achyranthes aspera* has a significant potential for use in pharmacology. Further studies of this plant species should be directed to detailed qualitative and in vivo studies of its medically active secondary metabolites in order to prepare natural pharmaceuticals of high value.

.

Reference

[1]. Badami S, Gupta M.K, and Suresh B, Antioxidant activity of ethanolic extract of Striga orobanchioides. J. Ethnopharmacol. 2003; 85: 227-230.

[2]. 2. Hazra B, Santana B, Nripendranath M. Antioxidant and free radicals scavenging activity of Spondias pinnata. J. BMC. 2008; 8: 63.

[3]. Jose A. Yuri. Antioxidant activity and total phenolics concentration in apple peel and flesh is determined by cultivar and agroclimatic growing regions in Chile Journal of Food, Agriculture & Environment 2009; 7 : 513-517.

[4]. .Valko M, Leibfritz D, Moncola J, Mazura M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. International Journal Biochemistry Cell Biology, 2007; 39, 44–84.

[5]. Huda-Faujan, N, Noriham, A, Norrakiah A. S, Babji A. S. Antioxidant activity of plants methanolic extracts containing phenolic compounds. African Journal of Biotechnology. 2009; Vol. 8 (3): pp. 484-489

[6]. Sumazian Y, Syahida A, Hakiman M, Maziah M, Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J. Med. Plant. Res. 2010; 4(10): 881-890.

[7]. T Sun; Ho CT. Antioxidant and Antibacterial Activities of Various Extracts and Fractions of Fresh Tea Leaves and Green Tea . Food Chemistry, 2005; 90: 743-749.

[8]. Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis, 2006; 19: 531-537.

[9]. Hirose M, Imaida K, Tamano S, Ito, N. Cancer chemoprevention by antioxidants. In "Food Phytochemicals for Cancer Prevention II". 1994; 122-132.

[10]. Girach RD, Khan ASA. Ethnomedicinal uses of Achyranthes aspera leaves in Orissa (India). Int J Pharmacogn.1992; 30:113-115.

[11]. Liersch Bkher. Achyranthes. In: Haensel R, Keller K, Rimpler G, Schneider G (eds) Hagers Handbuch der Pharmazeutischen Praxis. V. Springer-Verlag. Berlin7, 1992; 54-59.

[12]. Jain SK. Dictionary of Indian folk medicine and ethnobotany. Deep Publications, New Delhi, India.1991.

[13]. Bhom KH, Liersch R, Haensel R, Keller K, Rimpler H. Schneider (Eds.).G, Hagers Handbchder Pharmazeutischen Praxis Springer Verlag. Berlin. 1992;5,54-59.

[14]. Vasudeva N, Sharma SK. Estrogenic and pregnancy interceptory effects of Achyranthes aspera Linn. Root. J.Ethanopharmacol. 2006;179.

[15]. Paul D, Bera S, Jana D, Maiti R. Ghosh.D. In Vitro determination of the contraceptive spermicidal activity of a composite extract of Achyranthes aspera and Stephania hernandifolia on human semen. Contraception. 2006; 73:284-288.

[16]. Mohammad SA Javed I. evaluation of hypoglycemic effect of Achyranthus aspera in normal and alloxan diabetic rabbit. Journal of Ethanopharmacology 1991; 31(1):49-57.

[17]. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002; 10: 178-182

[18]. McDonald S, Prenzler PD, Antolovich M, Robards K Phenoliccontent and antioxidant activity of olive extracts. Food Chem. 2001; 73:73-84.

[19]. Koleva II, Van Beek TA, Linssen JPH, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 2002; 13: 8-12.

[20]. Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate frombrown alga Ecklonia cava. Food Chem. Toxicol. 2006; 44: 1065-1074.

[21]. Yen, G. C., & Duh, P. D. Antioxidative properties of methanolic extracts from peanut hulls. Journal of the American Oil Chemists Society. 1993; 70, 383–386

[22]. Wagner H, Bladt S, zgainski E M. Plant drug analysis. A thin layer chromatography atlas, springer. germany, 2nd edn, 1984.

[23]. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition Critical Reviews in Food Science and Nutrition. 1992; 32: 67-103.

[24]. Halliwell B Free radicals, antioxidants and human disease: curiosity, cause, or consequence Lancet 1994; 344: 721-724.