June 2009 Volume 5 Issue 2

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 5(2), 2009 [78-80]

Evaluation of *in-vitro* antioxidant activity of methanol extract of *Asystasia gangetica* (L).T (Chinese violet)

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Received: 18th April, 2009 ; Accepted: 23rd April, 2009

ABSTRACT

The present investigation evaluates the antioxidant activity of methanolic extract of leaves of *Asystasia gangetica* (L).T. (Acanthaceae) in various *in vitro* models. Besides, total phenolic was tested using Folin Ciocalteau reagent was investigated by method given by Liu et al. The methanolic extract of *Asystasia gangetica* shown significant antioxidant activity ($p<0.05$). The DPPH radical scavenging, nitric oxide scavenging and superoxide scavenging activity of methanolic extract have IC_{50} value 179.67, 33.43, 17.106 respectively. The effect of methanol extract on reducing power of the extract was studied according to the reaction of Fe^{+3} to Fe^{+2}. The reducing power of the extract increased with the increasing amount of the concentration. The *in vitro* studies clearly indicate that the methanol extract of *Asystasia gangetica* has significant antioxidant activity.

KEYWORDS

Asystasia gangetica;
Antioxidant activity;
DPPH method;
Superoxide scavenging activity;
Nitric oxide scavenging activity;
Reducing power assay.

INTRODUCTION

Asystasia gangetica (L).T. (Chinese violet) is a rapidly growing perennial shrubby herb mainly distributed in north India, which grows to 10 m height, at an altitude 300m[1] neutralized in some waste areas[2]. Leaves are opposite petioles, flowers are pale purple blue to violet or lime white in colour, capsules are 2.5-3.5 cm long with wide base and the seeds are 5 mm in diameter. It is mainly used in mild hypoglycaemia, anticancer against epidermoid carcinoma of nasopharynx. The juice of the plant is also used as an anthelmintic[3]. It is used in swelling and rheumatism, as a remedy for gonorrhea and ear disease. It is used as folk remedy for the treatment of diabetes mellitus in parts of south India[4]. It is evaluated for anti-asthmatic activity[5].

Asystasia gangetica reported to contain biologically active substances such as carbohydrates, proteins, alkaloids, tannins, steroidal aglycones, saponins, flavonoids and triterpenoids.

Study was undertaken to investigate the *in-vitro* antioxidant of leaves of *Asystasia gangetica.*

EXPERIMENTAL

Plant material and preparation of extract

Leaves of *Asystasia gangetica* were collected from Siruvani forest Coimbatore district. The plant was authenticated by Dr. P.Venu joint director, botanical survey of India, Tamil nadu (voucher specimen no BSI/ SC/ 5/23/05-06/Tech-538). The air-dried leaves of *Asystasia gangetica* were pulverized and the powdered material was extracted with methanol (70 %) by cold maceration. The extract was concentrated on a rotary vacuum evaporator, which gave a greenish-brown

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yield (3.65% w/w). The proximate phytochemical analysis of methanol extracts shows presence of flavonoids, proteins and carbohydrates, alkaloids, tannins, saponins[6].

Determination of DPPH radical scavenging activity[7]

1ml different conc. of extract solution and standard were taken in different vials. To this 5ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37^0C for 20min. Measure the absorbance against methanol as blank at 517nm. Take absorbance of the DPPH as control, Percent antiradical activity can be calculated by using following formula

% Antiradical activity = Control Abs- sample A / Control Abs. × 100

Reducing power assay[8]

1 ml of 10, 20, 30, 40, 50μg/ml of extract solution was mixed with 2.5ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50^0C for 20min 2.5ml of TCA was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5ml of upper layer solution was taken and mixed with 2.5ml distilled water and 0.5ml of ferric chloride solution and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Nitric oxide scavenging activity[9]

Different concentrations of sample solution were prepared in 100 ml volumetric flask. To this 0.1489 g of sodium nitroprusside (final concentration 5 mM) was added and kept for incubation. At different time 5.6 ml was taken, 0.2 ml was this reagent A was added and kept for incubation at 30^0C for 10 mins. After incubation 0.2 ml of Griess reagent was added and kept for incubation at 30^0C for 20 minutes. After incubation absorbance was measured at 542 nm against blank.

% NO Scavenging activity = Control Abs- sample A / Control Abs. × 100

Superoxide scavenging activity[10]

Reaction mixture with a final volume of 1ml per tubes was prepared with 50mM potassium phosphate pH 7.4 containing 1mM EDTA, 100μM hypoxanthine,100mM NBT 0.066 per tube of xanthine oxidase and test extract in 10μl of saline. The subsequent rate of NBT reduction was determined on the basis of spectrophotometric determination of absorbance at 560nm.

% inhibition = $[(A_0-A_1)/A_0] \times 100$

Estimation of Total phenolic content

Total phenolic in the methanolic extracts were determined with Folin-Ciocalteau reagent according to the method of Liu et al.[11] using gallic acid as standard phenolic compound. 1.0 ml of extract solution containing 1.0 g extract in volumetric flask was diluted with 46 ml of distilled water.1.0 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3minutes later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue colour that developed was read at 760 nm. The concentration of total phenol was expressed as mg/g of dry extract[13]. The concentration of total phenolic compound in the extract was determined as μg of gallic acid equivalent using an equation obtained from the standard gallic acid.

RESULT AND DISSCUSSION

The methanolic extract demonstrates potent antioxidant activity in different *in vitro* models. The DPPH radical scavenging, nitric oxide scavenging and superoxide scavenging activity of methanolic extract have IC_{50} value 179.67, 33.43, 17.106 respectively. Also extract possesses potent reducing power.

In-vitro antioxidant was carried on methanolic extract with various models. In addition the methanolic extract found to contain a noticeable amount of total phenol. The total phenolic content of *Asystasia gangetica* was found to be 0.902 mg/ml, which play major role in controlling antioxidants[12].

The production of reactive oxygen species in plants is well documented and these are key components in plant defensive responses. Most plants constitutively synthesize phenylpropanoids including flavonoids and hydroxycinnamic acids. However, accumulation of phenolics in plants can be induced by abiotic and biotic stresses[13-15]. Since the reduced forms of phytophenolics are powerful antioxidants equivalent to ascorbate, the plant community is a logical starting point for the search of antioxidant compounds. While studying antioxidant potential of *Asystasia gangetica,* it was found that a

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TABLE 1: Anti-radical activity of methanolic extract of *Asystasia gangetica*

Sr. no.	Control(mcg)	Mean ± SEM	% Antiradical activity
ASC	10	0.3411±0.00043	59.98
ASC	20	0.2508±0.000026	70.51
ASC	30	0.2273±0.00011	73.28
ASC	40	0.1954±0.00045	76.99
ASC	50	0.1949±0.04303	74.20
AGM	10	0.2582± 0.00044	72.16
AGM	20	0.2471±0.000116	70.95
AGM	30	0.2365±0.000693	69.57
AGM	40	0.2479± 0.00033	64.21
AGM	50	0.3066±0.00066	63.94

ASC-Ascorbic acid, AGM- methanolic extract of *Asystasia gangetica*, Absorbance of blank = 0.8512

TABLE 2: Reducing power assay of methanolic extract of *Asystasia gangetica*.

Sr. no.	Control (mcg)	Mean ± SEM
BHA	25	0.0716±0.0017
BHA	50	0.3476±0.0014
BHA	75	0.5516±0.0014
BHA	100	1.01±0.00118
BHA	125	0.024±0.0011
AGM	10	0.0369±0.00176
AGM	20	0.0418±0.00033
AGM	30	0.0446±0.00033
AGM	40	0.0574±0.0019
AGM	50	0.0719± 0.00090

AGM- methanolic extract of *Asystasia gangetica*, BHA- Butylated hydroxyl anisole

TABLE 3: Nitric oxide scavenging activity of of *Asystasia gangetica*

Sr. no.	Standard BHA (mcg)	Mean ± SEM	% Antiradical activity
BHA	50	0.9077±0.0021	51.26
BHA	100	0.983±0.00113	47.12
BHA	150	0.9717±0.00093	47.82
BHA	200	0.944±0.0025	49.16
BHA	250	1.025±0.0025	45.07
BHA	300	1.158±0.0035	38.08
AGM	10	1.387±0.0075	24.95
AGM	20	1.65±0.0032	10.75
AGM	30	0.6003±0.0057	67.67
AGM	40	0.687±0.00208	63.25
AGM	50	0.7421±0.00057	62.50
AGM	60	0.8132±0.000832	57.53

AGM- methanolic extract of *Asystasia gangetica*, BHA- butylated hydroxyl anisole

plant usually has higher antioxidant potential.

Thus the result of this study shows that the methanolic extract can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

TABLE 4 : Superoxide radical scavenging activity of of *Asystasia gangetica*

Sr. no.	Standard(mcg)	Mean ± SEM	% ALP
BHA	25	0.3686±0.0006	60
BHA	50	0.3468±0.0092	65
BHA	100	0.3355±0.000551	67
BHA	200	0.294±0.000116	72
AGM	10	0.2807±0.000153	73.58
AGM	20	0.287±0.00035	73.07
AGM	30	0.2538±0.0030	75.22
AGM	40	0.2453±0.00033	77.76
AGM	50	0.221±0.0010	78.6

AGM- methanolic extract of *Asystasia gangetica*, BHA-butylated hydroxyl anisole

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