ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 3, 2015

ISSN - 0974-2441

Research Article

ANTIOXIDANT ACTIVITY OF *MYXOPYRUM SMILACIFOLIUM* BLUME

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Received: 24 January 2015, Revised and Accepted: 03 February 2015

ABSTRACT

Objective: To investigate the antioxidant activity of ethanol and aqueous extracts of *Myxopyrum smilacifolium* Blume.

Methods: Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, nitric oxide radical and superoxide scavenging activity, using different concentrations (10, 25, 50, 100 µg/ml).

Results: The extracts showed significant activity as compared to control; but comparatively less than the ascorbic acid.

Conclusion: The extracts of myxopyrum smilacifolium blume showed antioxidant activities.

Keywords: Antioxidant, *Myxopyrum smilacifolium,* 2,2-diphenyl-1-picrylhydrazyl, Reducing power, Nitric oxide radical.

INTRODUCTION

A large number of medicinal plants available in nature and an impressive number of modern drugs have been isolated from natural sources like plant, mainly based on their use in traditional medicine. Free radicals are the main culprit in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources [1]. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism [2]. Free radical oxidative stress caused a wide variety of clinical disorders [3]. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, alzheimer's disease, and cancer[4].

M. smilacifolium Blume (Family-Oleaceae) is an important medicinal plant widely used in the indigenous system of medicines in India. The leaves are acrid, astringent, sweet, anodyne, febrifuge, thermogenic, and tonic. These are useful in vitiated conditions of kapha, vata, cough, asthma, nostalgia, rheumatism, consumption, otopathy, fever, neuropathy, cuts and wounds [5,6]. Earlier the plant has been studied for its antimicrobial [6], wound healing [7] and anti-inflammatory activity [8].

MATERIALS AND METHODS

Collection of plant material

The leaves of *M. Smilacifolium* Blume were collected from Agricultural University, Odakkali, Perumbavoor Ernakulam district, Kerala (India) in the month of September 2013 and authenticated by Dr. Harikrishnan E, Assistant professor, Department of Botany, Payyanur College, Kannur, Kerala. The leaves were dried in shade at room temperature. The dried leaves of *M. smilacifolium* were pulverized in a mechanical grinder to obtain coarse powder.

Preparation of plant extracts

Ethanol extract: The shade dried powdered leaves (500 g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The ethanol was concentrated in vaccum to a syrupy consistency. The percentage yield of extract was found to be 3.75%.

Aqueous extract: The aqueous extract was prepared using fresh powder by the maceration process. A volume of 100 g of the powdered drug was taken in a 2000 ml conical flask with 500 ml of distilled water, and 10 ml chloroform is added as a preservative. It was extracted up to 7 days with daily 2 hrs stirring with the help of the mechanical stirrer. After 7 days of the process, the extract was filtered through the muslin cloth and the marc was is discarded and airtight container in its filtrate dried under hot air oven at 45° C to semisolid mass. These were stored in the refrigerator below 10°C. The percentage yield of extract was found to be 5%.

Determination of *in-vitro* antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with a hydrogen donor changes to yellow in color. The free radical scavenging activity was measured by DPPH assay using the method of Blois [9]. 0.1 mM solution of DPPH in methanol was prepared, and 1 ml of this solution was added to 3 ml of various concentrations of EEMS and AEMS (10, 25, 50 and 100 µg). After 30 minutes, absorbance was measured at 517 nm. BHT (25 µg) was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples[10].

Reducing power

The reducing power was determined according to the method of Oyaizu[11]. Different concentrations of EEMS and AEMS (10, 25, 50 and 100 µg) in 1ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 500°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Sodium metabisulfite was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicates increase in reducing power.

The percentage reducing power was calculated using the formula:

$$\text{Percentage increase in absorbance} = \frac{\text{Control OD} - \text{Test OD}}{\text{ControlOD}} \times 100$$

Superoxide anion scavenging activity

Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them when supplied at concentrations greater than those in normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of $H_2O_2^+$ the superoxide radical, $O_2^{\bullet}$. This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity was done using the method explained by Nishimiki [12]. About 1 ml of nitro blue tetrazolium (NBT) solution containing 156 µM NBT which is dissolved in 1.0 ml of phosphate buffer (100 mM, pH 7.4), 1 ml of NADH solution containing 468 µM of NADH which is dissolved in 1 ml of phosphate buffer (100 mM, pH 7.4) and 0.1 ml of various concentration of EEMS and AEMS and the reference compounds (10, 25, 50 and 100 µg) were mixed and the reaction started by adding 100 µl of phenazine methosulfate (PMS) solution containing 60 µM of PMS 100 µl of phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 250°C for 5 minutes, and the absorbance at 560 nm was measured against the control samples. Vitamin C used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage decrease in absorbance was calculated [13].

Hydroxyl radical scavenging activity

In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical, $OH^{\bullet}$, this can attack and destroy almost all known biochemical system. Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogeneous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like $O_2^{\bullet}$, $OH^{\bullet}$ and H_2O_2, which are generated in solution from auto-oxidation of phenyl hydrazine. This forms the basis of this experiment. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radical generated by Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao [14]. The reaction mixture contained in a final volume of 1.0 ml, 100 µl of 2-deoxy-2-ribose (28 mM in KH_2PO_4-KOH buffer, 20 mM, pH 7.4), various concentrations of EEMS and AEMS(10, 25, 50 and 100 µg) and the reference compound sodium metabisulfite (25 µg) in KH_2PO_4-KOH buffer (20 mM, pH 7.4), 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of 1.0 mM H_2O_2 and 100 µl of 1.0 mM ascorbic acid was incubated at 370°C for 1h. A volume of 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added to the test tubes and incubated at 100°C for 20 mintues. After cooling, absorbance was measured at 532 nm.

RESULTS AND DISCUSSION

DPPH assay observed that the ethanol and aqueous extract of *M. smilacifolium* have dose-dependent increase in the DPPH radical scavenging activity against BHT (99.79%) (Tables 1 and 2). A volume of 100µg of ethanol extract has shown maximum scavenging activity (66.31%) (Table 1). Ethanol extract has shown more activity than aqueous extract.

Reducing power of both the extracts was concentration dependent. *M. smilacifolium* at high concentration showed higher reducing power which is lower than 25 µg sodium metabisulfite (71.96%) (Table 1), 100 µg of EEMS and AEMS have shown maximum reducing power at (62.92%) and (53.58%) respectively (Tables 1 and 2).

Superoxide anion scavenging activity was observed where EEMS and AEMS demonstrated dose-dependent increase (Tables 1 and 2). Vitamin C showed superoxide anion scavenging activity (76.83%) at 25 µg (Table 1) whereas AEMS has shown 65.54% activity at 100 µg (Table 2).

Table 1: Effect of ethanolic extract of *M. smilacifolium* on free radical scavenging activity using different models

Sl. No	Conc. µg/ml	% Scavenging			
		DPPH	Reducing power	Superoxide anion	Hydroxyl radical
1.	Standard 25µg	99.79	71.96	76.83	72.58
2.	10	12.95	34.89	39.88	44.54
3.	25	32.63	42.67	49.12	49.53
4.	50	32.63	48.90	63.19	52.02
5.	100	66.31	62.92	67.59	62.30

M. smilacifolium: Myxopyrum smilacifolium, DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2: Effect of Aqueous extract of *Myxopyrum smilacifolium* on free radical Scavenging activity using different models

Sl. No	Conc. µg/ml	% Scavenging			
		DPPH	Reducing power	Superoxide anion	Hydroxyl radical
1.	10	26.23	08.4	44.09	34.57
2.	25	29.62	33.64	51.79	43.30
3.	50	50.15	42.67	58.94	60.43
4.	100	64.84	53.58	65.54	68.84

M. smilacifolium: Myxopyrum smilacifolium, DPPH: 2,2-diphenyl-1-picrylhydrazyl

A volume of 25µg of sodium metabisulfite was used as a standard for hydroxyl radical scavenging activity (72.58%). A volume of 100 µg EEMS and AEMS have shown significant hydroxyl radical scavenging activity 62.30% and 68.84% respectively (Tables 1 and 2).

CONCLUSION

In the present study, results indicated that the ethanol and aqueous extract of *M. smilacifolium* possess antioxidant activity. *M. smilacifolium* showed presence of several bioactive compound *viz*. terpenoids, tannins, volatile oil, phenolic compounds and saponins. This encourages antioxidant studies. Further investigations are required to find out active component of the extract.

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