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ANTIOXIDANT PONTENTIAL OF METHANOLIC EXTRACT OF ROOT, CALLUS AND FRUIT EXTRACTS OF *MYXOPYRUM SMILACIFOLIUM* BLUME

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ABSTRACT

The aim of the present study was to compare the anti-oxidant efficacy of methanolic extract of root, callus and fruit of *Myxopyrum smilacifolium* Blume. The antioxidant activity was determined using three assays namely, Hydroxyl radical scavenging assay (HRS), 2, 2-diphenyl -1-picrylhydrazyl (DPPH) and Nitric Oxide scavenging assay (NOS). Its IC50 values were also determined. All the extracts showed considerable scavenging activity and significant activity against DPPH. Maximum scavenging activity was shown by root extracts against DPPH and NOS while fruit extracts against HRS. From the study it was concluded that the plant might be promising as a curative for many diseases associated with free radicals.

Key Words: Callus, Antioxidant, DPPH, IC50.

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al*., 2006). Plant derived chemicals are a promise for it to be used as the active ingredients of modern medicine or as the lead compounds for new drug discovery.

Myxopyrum smilacifolium is a large woody climbing shrub belonging to the family Oleaceae. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicine. The roots are used to treat various diseases like scabies, cough, rheumatism, fever, cuts and wounds. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, fever, otopathy, neuropathy and cuts and wounds (Warrier, 1996). Pharmacognostical evaluation has been made for the plant and reported for the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, phenols, tannins, and saponins, antimicrobial study has been carried out in leaves (Gopalakrishnan *et al*., 2012). Previous studies have shown the presence of triterpenoid ursolic acid in leaves (Sudharmini and Ashalatha, 2008) and the iridoid glycoside myxopyroside (Franzyk, 2001).

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen generated as by-products of cellular metabolism, primarily in the mitochondria. ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress

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(Devasagayam *et al*., 2004). Such a state of "oxidative stress" is thought to contribute to the pathogenesis of a number of human diseases (Thannickal VJ and Fanburg BL, 2000). Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease (Nunomura *et al*., 2006), Parkinson's disease (Wood *et al*., 2005) the pathologies caused by diabetes (Giugliano *et al*., 1996), rheumatoid arthritis (Hitchon and El-Gabalawy, 2004) and neurodegeneration in motor neuron diseases (Cookson and Shaw, 1999).

Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Zengin *et al*., 2011). Plant based medicines are safer than their synthetic counterparts although their toxicity levels has to be assessed before approval. The advantage of plant derived antioxidants is that it has no toxic effects as compared to synthetic antioxidants such as Butylated Hydroxy Toluene (BHT).

The present study was carried out to in order to have a comparative study on the antioxidant efficacy between the methanolic root, callus and fruit extracts. Three common antioxidant assays *viz:* Hydroxyl radical scavenging activity, DPPH assay and Nitric oxide scavenging activity were employed in the present study.

MATERIALS AND METHODS

Collection and processing of plant material

Fresh plant roots of *M. smilacifolium* were collected from Botanical garden, Dept. of Botany, University of Kerala, Kariavattom. The roots were washed thoroughly with tap water followed by sterile distilled water. Shade dried roots were then crushed to coarse powder and were stored at room temperature in air tight containers.

For callus induction surface sterilized internode explants were first inoculated in MS medium supplemented with 0.1% 2, 4 Dichlorophenoxy acetic acid (2,4-D) and then sub-cultured on to same medium supplemented with a combination of 0.1% 2,4-D and 1.0 % Benzyl amino purine (BAP). Four week old callus was collected and dried in hot air oven at 50^0C. Dried callus then powdered using mortar and pestle and stored in refrigerator.

Fresh matured fruits collected were washed thoroughly under running tap water, chopped into pieces and dried in shade. It was then crushed with mortar and pestle and stored in refrigerator.

Extraction

Powdered root, callus and fruit of *M.smilacifolium* were extracted using methanol in soxhlet apparatus for 12 hrs. The extracts were then filtered through Whatmann No.1filter paper and concentrated using vacuum evaporator. The extract value calculated and then stored in refrigerator for further use.

Antioxidant activity

All the three extracts were subjected to antioxidant activity assays viz: Hydroxyl radical scavenging activity, DPPH assay (2, 2-diphenyl -1-picrylhydrazyl) and Nitric oxide scavenging assay for comparing their activity.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity (Ilavarasan *et al*., 2005) assay is based on the quantification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe3+ - ascorbate- EDTA –H2O2 system (Fenton reaction). The reaction mixture contained Hydroxyl radical scavenging activity (Ilavarasan *et al*., 2005) assay is based on the quantification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe3+ - ascorbate- EDTA –H2O2 system (Fenton reaction). The reaction mixture contained in the final volume of 1 mL.2 deoxy 2 ribose (2.8mM) KH2PO4—KOH buffer (20 mM pH 7.4), FeCl3 (100µm), EDTA (100µm), H2O2 (1.0mM), ascorbic acid (100µm) and various concentrations (125-2000µg/ml) of the test sample. After incubation for 1hour at 37°C, 0.5 ml of the reaction mixture was added to 1ml of 2.8% TCA, then 1ml aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution.

$$\% \text{ activity} = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} X100$$

DPPH assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang *et al.,* 2001. The principal of this assay is that 1,1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm .Ascorbic acid (10mg/ml DMSO) was used as reference. 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

Different volumes (1.25-20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

$$\% \text{ activity} = \frac{OD\ of\ control - OD\ of\ test}{OD\ of control} X100$$

Nitric oxide scavenging activity

Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO, its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH and NO (Ravichandran and Mubarak, 2014). The procedure is based on the principle that, sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO. may lead to tissue damage.

Nitric oxide scavenging activity(Garrat, 1964) was measured spectrophotometrically. Sodium nitro prusside (5mmolL^{-1})in phosphate buffered saline pH 7.4,was mixed with different concentration of the extract (125-2000µg mL^{-1})prepared in methanol and incubated at 25°C for 30minutes .A control without the test compound, but an equivalent amount of methanol was taken. After 30minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent (1% sulphanilamide,2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphathyl ethylene diamine dihydrochloride was measured at 546nm and the percentage scavenging activity was measured with reference to the standard.

$$\% \text{ activity} = \frac{OD\ of\ control - OD\ of\ test}{OD\ of control} X100$$

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and SD was calculated.

Antioxidant Activity

The result of the antioxidant activity investigated is as given on table 1 and 2. Table 1 shows both hydroxyl and nitric oxide radical scavenging assay. Table 3 shows DPPH scavenging assay. The IC50 values obtained are also shown in the tables.

Table 1. Hydroxyl radical and nitric oxide scavenging Assay of extracts

Methods	Methanolic Extract	Concentration µg/% Inhibition					IC50
		125	250	500	1000	2000	
Hydroxyl radical Scavenging Assay	Root	17.29±0.78	26.05±0.56	29.14±1.04	48.60±0.87	66.52±1.24	1050
	Callus	11.27±0.91	23.36±0.75	27.78±1.21	41.36±0.67	59.18±1.24	1475
	Fruit	19.41±1.12	29.47±0.64	33.70±0.94	52.10±1.14	71.42±0.49	950
	Ascorbic Acid (Standard)	53.16±0.23	55.33±0.29	64±0.18	70.36±0.52	77.71±0.41	-
Nitric Oxide Scavenging Assay	Root	31.69±1.04	45.21±0.72	49.13±0.67	61.30±0.49	65.65±1.03	525
	Callus	24.69±1.03	32.82±0.34	36.26±0.21	48.60±0.39	56.29±0.62	1250
	Fruit	23.70±1.02	30.52±0.57	49.43±0.92	56.30±1.09	61.04±0.79	575
	Gallic Acid (Standard)	47.82±0.43	56.52±0.41	60.86±0.57	65.21±0.29	78.26±0.62	-

Each value represents the mean ± SD done in triplicate.

Table 2. DPPH Assay of root, callus and fruit extracts

Method	Methanolic Extracts	Concentration µg/% inhibition					IC50
DPPH Assay		12.5	25	50	100	200	
	Root	27.45±0.96	46.56±1.15	53.67±0.74	69.48±1.03	81.92±0.69	37.5
	Callus	24.09±0.59	29.84±0.35	41.20±0.79	49.82±1.23	64.86±0.71	47.5
	Fruit	21.81±0.62	32.49±1.21	46.16±0.43	52.28±0.51	68.98±0.83	87.5
	Ascorbic acid (Standard)	52.57±0.23	74.95±0.56	82.61±0.78	89.38±0.25	91.45±0.41	-

Each value represents the mean ± SD done in triplicate.

DISCUSSION

Previous studies have shown that *Myxopyrum smilacifolium* is medicinally important plant as it is rich in many phytoconstituents. So the present study was carried out in order to compare the antioxidant efficacy of methanolic extracts of root, callus and fruits. Reactive Oxygen Species has considerable interest because of its role in several pathological conditions. The plant might contain an appreciable amount of reductants which may react with the free radicals to stabilize and terminate them from free radical chain reaction. Present study investigated the antioxidant activity of the three extracts using three standard free radical scavenging assays. Hydroxyl radical scavenging assay was shown maximum by Methanolic fruit extract followed by root extract. But the callus extract showed only moderate activity. But all the three samples showed an appreciable DPPH free radical scavenging activity with root extract the most potent followed by callus and fruit extracts. They showed a comparable activity with the standard. Nitric acid scavenging activity was shown maximum by root extract followed by fruit extract and finally callus extract. Taking into account of all the samples root extract and fruit extracts shows almost same free radical scavenging potential followed by callus extracts. Of the three free radical scavenging assays conducted DPPH scavenging was shown by three samples with root extracts having an IC50 value of 37.5. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that antioxidant activities that can be made therapeutically useful (Kanatt *et al*., 2007). This study reveals that tested plant materials have moderate to significant free radical scavenging activity thereby utilizing at as a good candidate in pharmaceutical industry for drug development after considering various parameters. Extracts showing positive results have a concentration dependent activity. The present study surely forms a basic for further studies to investigate the bioactive compounds, their isolation and structure elucidation.

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