Research Article

Free Radical Scavenging and Anti Protease Activity of Methanolic Extract of *Mitracarpus villosus* (SW)

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ABSTRACT

Mitracarpus villosus (Sw) is a herb from Rubiaceae family used in traditional medicine for the treatment of several diseases such as hepatic, skin and venereal diseases, diarrhea and dysentery. It has also been used to treat ringworm and eczema, fresh cuts, wounds and ulcer. The aerial parts of this plant have also been used to make lotion and skin ointment used for skin diseases and infections. In this study we can evaluate the In vitro antioxidant and antiprotease activity of the methanolic extracts from *Mitracarpus villosus* (Sw). The methods used for antioxidant potentials include DPPH, ABTS, Nitric oxide and Hydroxy radical scavenging assays. We can evaluate the anti-inflammatory potentials by anti-protease activity. Antioxidant assays were done by using the concentrations in the range 20 -100µg/ml and the results were compared with the standard ascorbic acid. The anti-protease activity was done using BAEE (N-benzoyl-L-arginine ethyl ester) as substrate and Phenyl methyl sulphonyl fluoride (PMSF), is used as standard. The methanolic extract of the *Mitracarpus villosus* (Sw) showed better activity for both antioxidant and anti protease assays.

Keywords: Antioxidants, Free radicals, Oxidative stress, Rubiaceae, Inflammation.

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INTRODUCTION

Free radicals are defined as an atom or molecule having unpaired electrons. They are electrically charged and their electron imbalance provides them highly reactive and capable of oxidizing lipids, protein, nucleic acids and carbohydrates. There are several oxygen-derived free radicals, which are formed in living organisms by both endogenously and exogenously[1]. Different agents of the environment such as pesticides, ionizing radiation, lead, cadmium, tobacco smoke, liquor, lead, UV light also generate free radicals. Excess production of free radicals induces oxidative damage to lipids, proteins, nucleic acids, which may lead to atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and other degenerative disorders [2]. The role of reactive oxygen species (ROS) and other oxidants in causing several diseases has brought the attention of scientists to an appreciation of antioxidants for preventing diseases and maintain human health.

Oxidative stress plays an important role in chronic inflammatory disorders. The generation of ROS in brain tissues results in neurodegeneration, memory loss, neuroinflammation and cell death[3]. Inflammation is activated by the release of chemical mediators from injured tissue and migrating cells[4]. There are several proteolytic enzymes, which act as essential modulators of inflammatory response. Proteolytic enzymes inhibit pathogenic immune complexes. When these are formed in excess, they cause several disorders like renal diseases, rheumatoid arthritis etc. Proteolytic enzymes break down proteins present in plasma and other cellular debris into smaller fragments at the site of the injured area. This led to an increased interest among scientists to evaluate the role of antioxidant therapy in inflammatory conditions.

Antioxidants inhibit or delays oxidative damage to a target molecule [5]. They scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quenchers [6]. They prevent cellular damage by reducing oxidative stress and provide a beneficial effect on human health. The free radicals formed in the body are scavenged by natural and synthetic antioxidants [7]. But the synthetic antioxidants are very toxic having side effects and are now replaced by natural ones for their safer needs [8]. The side effects of anti-inflammatory drugs are one of the major problems in developing medicine today. Therefore, the development of new and more powerful drugs from plant origin is used in India for the treatment of many diseases in the traditional system of medicine.

Mitracarpus villosus (Sw) is a herb from Rubiaceae family used in traditional medicine for the treatment of several diseases such as hepatic, skin and venereal diseases, diarrhoea and dysentery [9]. It has also been used to treat ringworm and eczema, fresh cuts, wounds and ulcer. The aerial parts of this plant have also been used to make lotion and skin ointment used for skin diseases and infections [10]. The plant leaves are used to cure Eczema, and the plant has antibacterial, antifungal activities[11]. Studies carried out to demonstrate that the plant possesses hepatoprotective properties[12]. From the literature survey and upon the

ethnomedical importance, present work focused on in-vitro anti-inflammatory and antioxidant activity of aerial parts of *Mitracarpus villosus* (Sw).

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Mitracarpus villosus (Sw) was collected from the rural areas of Wayanad in Kerala. The plant was identified and authenticated by the department of botany, Calicut University, Kerala. The aerial parts of the *Mitracarpus villosus* (Sw) were dried under shade and then powered by the mechanical grinder. The powder plant material was extracted with methanol using a Soxhlet extraction apparatus.

In vitro Anti-oxidant activity of the plant material

DPPH Radical Scavenging Activity

1,1- Diphenyl-2- picryl hydrazyl (DPPH) is a free radical for measuring antioxidant activity. The reaction mixture includes 2.8 ml 100µM DPPH in methanol and was added with 0.2 ml plant extract at different concentrations. The mixture was incubated for 30 minutes and the optical density was taken at 517nm. Ascorbic acid is used as a reference standard and methanol without sample along with DPPH was taken as control [13].

% of Scavenging = (A control-A Test)/A control X 100

ABTS Radical Scavenging Assay

The assay generates the oxidation of ABTS (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate]) to a nitrogen-centred radical cation, ABTS•. The stock solutions include 7.4 mM ABTS added with 2.6 mM potassium persulfate. The working solution is made by the addition of these solutions in equal amounts and then allowed to react for 12 hrs in dark. It was then diluted by adding 1ml ABTS solution mixed with 60 ml methanol to get an optical density of 1.1±0.02 at 734 nm. 150 µl of different concentrations (0.2 – 1.0 mg/ml) of solvent extracts of the plant material was allowed to react with 2850 µl of ABTS solution for 2 hours. The optical density was taken at 734 nm using spectrophotometer [14]. IC50 values were also calculated for the three extracts.

% scavenging = (Absorbance of control- Absorbance of Test)/Absorbance of control X 100.

Nitric Oxide Radical Scavenging Assay

Sodium nitroprusside in PBS was mixed with 3ml of different concentration of the extract and incubated at 25°C for 150 min. 0.5 ml of the samples were mixed with 0.5 ml of Griess reagent. Measure the absorbance at 546 nm [15]. The percentage scavenging of the nitric oxide radical was determined by using the formulae

(A control-A Test)/A control X 100.

Hydroxyl radical scavenging assay

The assay is based on the capacity of phytochemicals to compete with salicylic acid for hydroxy radicals. The mixture includes 1 ml 1.5mM FeSO4 added with 0.7 ml 6 mM hydrogen peroxide, 0.3 ml 20 mM sodium salicylate and 1ml of different concentrations of three solvent extracts of the plant material. The mixture is then incubated for 1 hour at 37°C and the optical density of the hydroxylated salicylate complex was measured at 562 nm. The standard used for the assay is ascorbic acid [16].

In vitro anti-protease activity

This protease inhibition assay was carried out using the spectrophotometric assay by Sigma Aldrich with slight modifications. Trypsin, the protease is used to determine the anti-protease activity of different solvent extracts of plant material. The assay was done on the basis of the hydrolysis of the substrate BAEE (N-benzoyl-L-arginine ethyl ester) at the ester linkage leads to an increase of optical density at 253nm. The mixture consisted of 200 µl trypsin, 200 µl test and incubates for 10 min. The reaction is started by adding 3ml substrate BAEE ((N-benzoyl-L-arginine ethyl ester) and optical density was determined at 253 nm. Phenyl methyl sulphonyl fluoride (PMSF), is used as standard.

$$\text{Inhibition (\%)} = (1\text{-}B/A) \times 100$$

Where A = Change in optical density of control, B = change in optical density of test solution.

RESULTS AND DISCUSSION

DPPH radical scavenging assay

DPPH assay is one of the methods for screening the antioxidant activity of plant extracts. Here the percentage of scavenging of the methanolic extract is 57.10 ± 1.82 at a maximum concentration of 100 µg/ml. The IC50 value of methanolic extract was found to be 87.56 µg/ml. This assay is used to measure the ability of antioxidants to scavenge the DPPH free radicals. The DPPH is a stable, synthetic-free radical, which receive electron easily from antioxidants to become stable of diamagnetic nature [17]. The methanolic extract show better scavenging activity for DPPH free radicals and these activities of antioxidants on DPPH radicals is due to their hydrogen donating capacity. These free radicals get decolourized by antioxidants present in the methanolic extract of *Mitracarpus villosus* (Sw). Among the other plant extracts, the methanolic extract of *Mitracarpus villosus* (Sw) exhibit higher DPPH radical scavenging activity, which may be due to the presence of polyphenolics and flavonoids present in the plant sample.

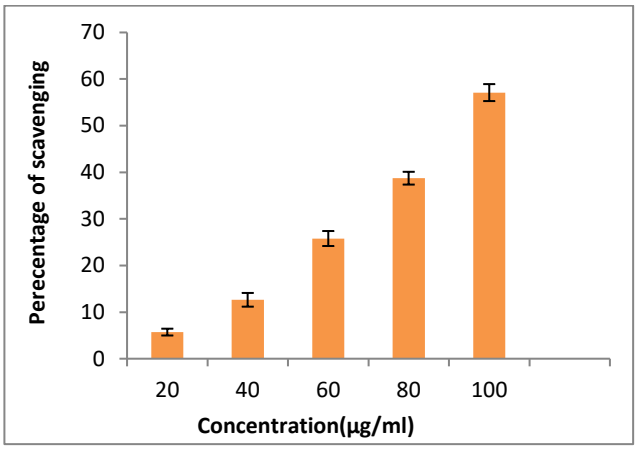

Figure 1: *In vitro* free radical scavenging effect of the Methanolic extract by DPPH method

ABTS radical scavenging assay

ABTS assay has been widely used to determine the antioxidant capacity of plant extracts. Here the percentage of scavenging of methanolic extract of Mitracarpus villosus (Sw) as 81.48 ± 0.83 at a high concentration of 100µg/ml. The IC50 value of methanolic extract was found to be 46.56µg/ml. In this assay, ABTS free radicals are scavenged by the antioxidants present in the methanolic extract of *Mitracarpus villosus* (Sw). The ABTS assay involves the oxidation of ABTS (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate]) to an intensely-coloured nitrogen-centred radical cation, ABTS•+, which has an absorption maxima at 734 nm. The presence of phytochemical compounds in the plant sample that inhibit the potassium persulfate activity may decrease the formation of ABTS•+radicals.

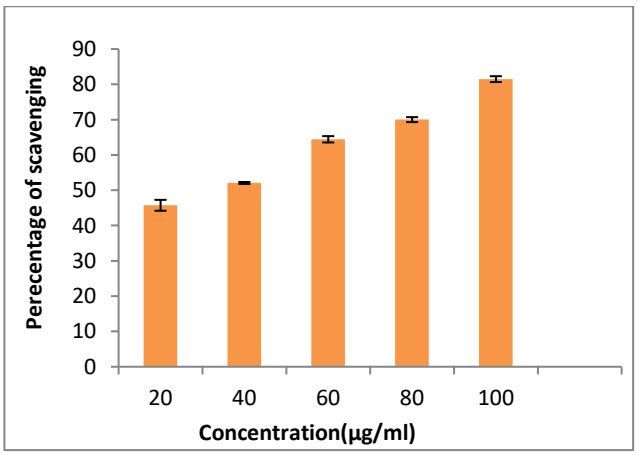

Figure 2: *In vitro* free radical scavenging effect of the methanolic extract by ABTS assay

Nitric oxide radical scavenging assay

A better scavenging activity for nitric oxide radicals was observed for the methanolic extract of *Mitracarpus villosus* (Sw) as 79.06 ± 1.87 at a high concentration of 100µg/ml. The IC50 value of methanolic extract was found to be 34.11µg/ml. Nitric oxide is involved in oxidative stress and various inflammatory processes and it acts as a potent inhibiter of several processes like the relaxation of smooth muscles, neuronal signalling and aggregation of blood platelets [18]. Scavenging of nitric oxide radical is based on the formation of nitric oxide. Sodium nitroprusside present in buffer saline reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. Here the methanolic extract of *Mitracarpus villosus* (Sw) reduced the amount of nitrite generated by the decomposition of sodium nitroprusside.

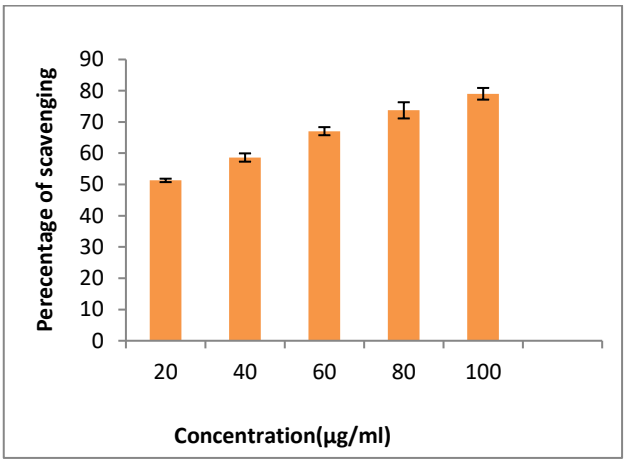

Figure 3: *In vitro* free radical scavenging effect of the extract by nitric oxide scavenging assay

Hydroxyl radical scavenging assay

Hydroxyl scavenging assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. A better scavenging activity for hydroxyl radicals was observed for the methanolic extract of *Mitracarpus villosus* (Sw) as 57.02 ± 1.87 at a high concentration of 100µg/ml. The IC50 value of methanolic extract was found to be 87.68µg/ml. The hydroxyl radicals are most reactive, which induces severe oxidative damage in adjacent biomolecules such as lipids, proteins and nucleic acids. The •OH scavenging` activity of methanolic extract of *Mitracarpus villosus* (Sw) was assessed by its ability to compete with salicylic acid for •OH radicals in the •OH generating/detecting system. In the present study, the hydroxyl radical-scavenging effect of *Mitracarpus villosus* (Sw) increased with increasing concentrations. The methanolic extract showed better scavenging activity for hydroxyl radicals.

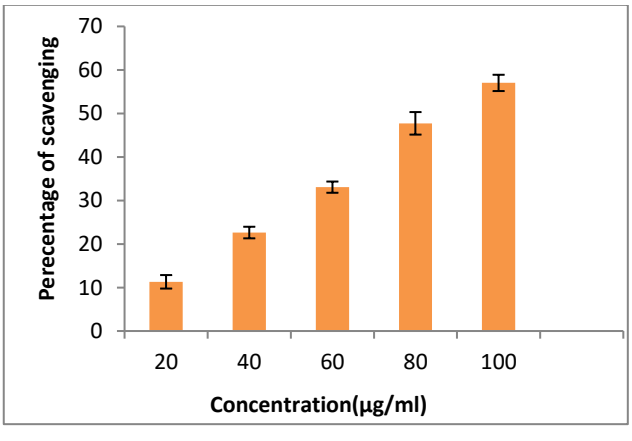

Figure 4: *In vitro* free radical scavenging effect of the extract by hydroxyl radical scavenging assay.

In vitro antiprotease activity

Neutrophiles contains a rich source of serine proteases and are localized at lysosomes and these proteases have a major role in the development of tissue damage during acute and chronic inflammatory conditions and a higher level of protection was provided by protease inhibitors [19]. These proteases are potential drug targets and their inhibitors have a major role in regulating protease activity. These inhibitors have medicinal properties in humans against inflammatory, immunological, respiratory disorders viral, parasitic infections, cancer etc. Anti protease activity was done using methanolic extracts of *Mitracarpus villosus* (Sw). Here the methanolic extract of the plant showed the highest inhibition of 65% and all the results were compared with the standard protease inhibitor (PMSF), which showed inhibition of 55.19 % at 100µg/ml.

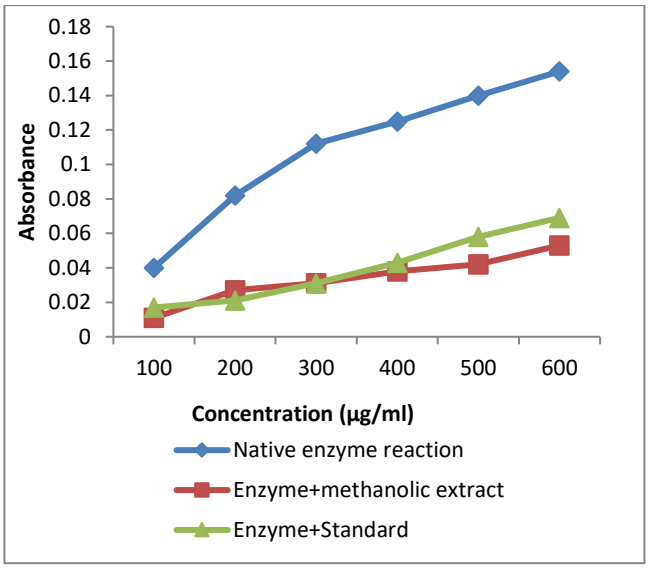

Figure 5: *In vitro* anti protease activity of methanolic extract of *Mitracarpus villosus* (Sw).

CONCLUSION

In the present study, results indicate that the methanol extract of *Mitracarpus villosus* (Sw) possess both antioxidant and antiprotease activities. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, phytosterols and polyphenolics. The methanolic extract serves as better scavengers of oxidative stress and antiprotease activity at a suitable concentration. From this study, we can estimate that methanol extract of *Mitracarpus Villosus* (Sw) has better antioxidant and antiprotease activity.

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