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# Evaluation of Antioxidant Activity of *Anisolmeles malabarica* R Br and *Clerodendrum serratum* L. Extracts against Rheumatism

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## ABSTRACT

The ethanolic extracts of the various parts of the plants namely *Anisolmeles malabarica* R.Br. (Lamiaceae) and *Clerodendrum serratum* L. (Verbenaceae) were investigated for their Antioxidant efficacy by DPPH and Nitric Oxide assay. Reactive Oxygen Species (ROS) and many of the free radicals are natural by-products of metabolism of drugs, environmental chemicals and other Xenobiotics as well as endogenous chemicals, especially stress hormones like adrenalin and noradrenalin. ROS has a high reactive potential and is responsible for many of the human diseases like diabetes, cancer, viral infections, cardiovascular diseases and inflammations and is known to cause oxidative damage to DNA, proteins and lipids in humans. The antioxidants present in the medicinal plants quench the free radicals by donating a hydrogen atom. The antioxidant activity of the medicinal plants namely, Anisolmeles malabarica and Clerodendrum serratum was estimated by using 2, 2-diphenyl-picryl-hydrazyl (DPPH) and Nitric Oxide assays. The present study aims at evaluating therapeutic potential of these medicinal plants due to their high free radical scavenging activity.

**Key words:** *Anisolmeles malabarica, Clerodendrum serratum*, Rheumatism, Antioxidants, DPPH, Nitric Oxide.

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## INTRODUCTION

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [1, 2]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurogenerative diseases and others [3-8]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [9-11]. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention [12].

In 1922, Goldschmidt and Renn [13] discovered the violet-coloured free stable radical 2,2-diphenyl-picrylhydrayl (DPPH), which now is used as ESR standard [14] and as colorimetric reagent [15, 16] for redox processes. Because DPPH can be kept indefinitely with little decomposition and because it neither dimerizes nor reacts with oxygen [17], it proved to be quite useful in a variety of investigations, such as polymerization inhibition or radical chemistry [18], the determination of antioxidant properties of amines, phenols or natural compounds (vitamins, plant extracts, medicinal drugs) and for inhibiting homolytic reactions [19-21]. DPPH is intensely violet like $KMnO_4$ and its reduced counterpart 2, 2-diphenyl-picrylhydrazine (DPPH-H) is orange yellow [18-21].

A survey of available literature shows that the antioxidant efficacies of different parts of the plants, namely Anisolmeles malabarica and Clerodendrum serratum, have not been used for the treatment of rheumatism. Hence, the present study has been undertaken to establish the antioxidant activity of these two medicinal plants by DPPH and Nitric Oxide assays.

## MATERILAS AND METHODS

### Plant materials

For the present study, the two following plants viz., Anisolmeles malabarica R.Br. belonging to the family Lamiaceae and Clerodendrum serratum L. belonging to the family Verbenaceae have been selected. These plants have been collected from Mysore, Nanjangud and surrounding areas where they were growing profusely. The plants have been identified and authenticated by experts from National Ayurveda Dietetics Research Institute, Bangalore (Ref. No. SMPU/NADRI/BNG/2010-11/550).

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## 1. Extraction of plant material

The plant materials were extracted with ethanol using sohxlet extraction appa ratus continuously for 16 hours [22]. For extraction, the dried plant material was used. Initially 400gms of material was packed in filter paper and loaded into the thimble of sohxlet apparatus. 2.5 liter of ethanol was poured into the flask (distilling pot) and the whole apparatus was set. The soxhlet extraction was performed for 12- 16 hours until the collected solvent in siphon tube appears to be clear. Later the extracted solvent was evaporated under reduced pressure to get solid/ semi solid extract. The extract was weighed, physical characters were noted. The percentage yield was calculated (Table 1).

**Table 1: Extraction of different parts of the plant material by ethanol**

| Sl. No | Sample | % yield |
|---|---|---|
| **1** | AML | 10.84 |
| **2** | AMA | 10.62 |
| **3** | AMR | 22.00 |
| **4** | CSA | 1.35 |
| **5** | CSR | 15.08 |

AML- Anisolmeles malabarica Leaves; AMA- Anisolmeles malabarica Aerial Parts; AMR- Anisolmeles malabarica Roots; CSA- Clerodendrum serratum Aerial Parts and CSR- Clerodendrum serratum Roots

## 2. Antioxidant studies

### i) DPPH Assay [23]

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

**Reagents**

**2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 μM)**: 22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 μM DPPH solution.

**Preparation of test solutions**:

21 mg each of the extracts was dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.

**Preparation of standard solutions:**

10 mg each of ascorbic acid and rutin were separately weighed and dissolved in 0.95 ml of Dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

**Procedure:**

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 µg/ml. The plates were incubated at 37$^{o}$ C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

**ii) Scavenging of Nitric Oxide radical [24]**

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess Ilosvay reaction. In the present investigation, Griess Ilosvay reagent is modified by using Naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm**.**

**Reagents**

- **Sodium nitroprusside solution**:

Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

- **Naphthyl ethylene diamine dihydrochloride (NEDD, 0.1%)**:

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

- **Sulphanilic acid (0.33% w/v) reagent**:

Weighed accurately 0.33 g of sulphanilic acid and dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.

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**Preparation of sample solutions:**

The sample solution was prepared as described in DPPH assay.

**Preparation of standard solutions:**

Weighed accurately 10 mg of ascorbic acid and rutin and dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

**Procedure:**

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25$^{o}$ C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm.

## RESULTS AND DISCUSSION

The test drug Clerodendrum serratum aerial parts extract showed good antioxidant properties against DPPH and Nitric oxide radical whereas Clerodendrum serratum roots extract showed good antioxidant properties against DPPH when compared to other extracts tested.

Scavenging activity of free radicals of 2, 2-diphenyl-picryl-hydrazyl (DPPH) and Nitric Oxide assays have been widely used to evaluate the antioxidant activity of many of the natural products from plants. Plant extracts from different parts of the two medicinal plants viz., Anisolmeles malabarica and Clerodendrum serratum listed in Table 1 were prepared for investigation for their antioxidant activities. Rutin was employed as the reference compound for the antioxidant and Nitric Oxide radical scavenging studies. The Clerodendrum serratum aerial parts extract exhibited strong DPPH and Nitric Oxide radical scavenging activity with the determined IC50 values 13.07 ± 0.34 and 142.30 ± 3.06, whereas the Clerodendrum serratum roots showed satisfactory activity against DPPH with the IC50 value of 25.80 ± 0.89 when compared to rest of the extracts tested (Table 2).

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Table 2: Antioxidant activity of the different parts of the plant extracts

| Sl. No | Samples | $IC_{50}$ values ± SE µg/ml* by methods | |
|---|---|---|---|
| | | DPPH | Nitric oxide |
| 1 | AML | 61.30 ± 0.67 | 995.00 ± 2.88 |
| 2 | AMA | 47.40 ± 1.02 | 691.70 ± 4.4 |
| 3 | AMR | 231.00 ± 0.57 | <1000 |
| 4 | CSA | 13.07 ± 0.34 | 142.30 ± 3.06 |
| 5 | CSR | 25.80 ± 0.89 | >1000 |
| Standards | | | |
| 6 | Rutin | 3.91 ± 0.10 | 65.44 ± 1.56 |

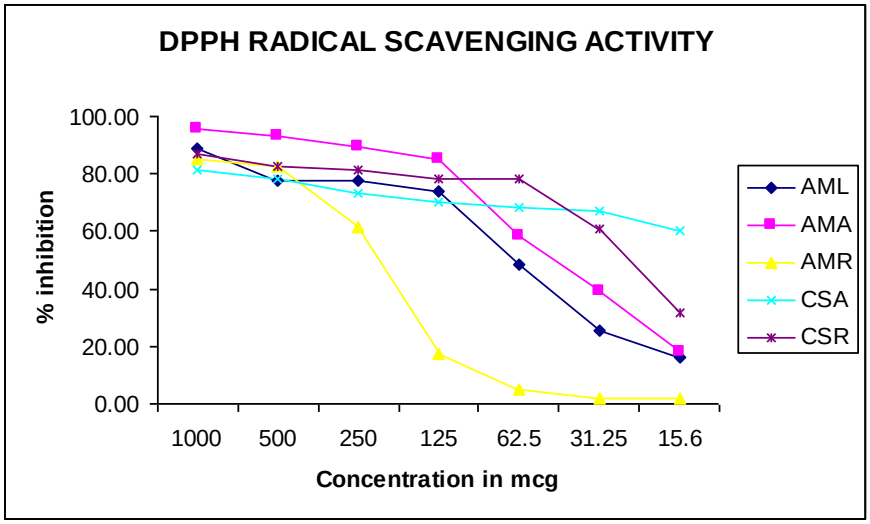


Figure 1: Plot of $IC_{50}$ values for the DPPH Radical Scavenging activity of different parts of the plants namely Anisomeles malabarica and Clerodendrum serratum. (Data from Table 2)

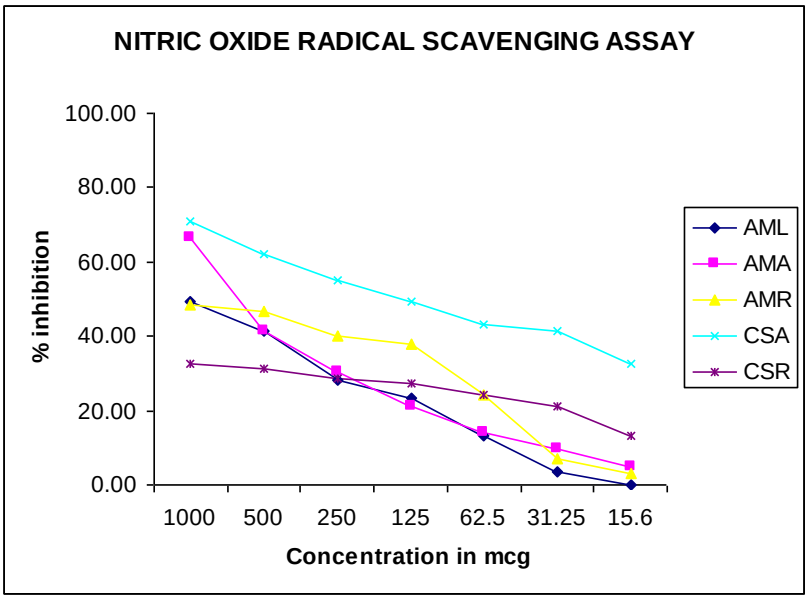


Figure 2: Plot of $IC_{50}$ values for the Nitric Oxide Radical Scavenging assay of different parts of the plants namely Anisomeles malabarica and Clerodendrum serratum. (Data from Table 2)

Phytochemical screening of the aerial parts of the plants of Anisolmeles malabarica and Clerodendrum serratum revealed the presence of flavonoids and these natural products are responsible for anti-inflammatory and antioxidant activity. The results of the present study support the folklore use of both these plants in rheumatic diseases. Flavonoids have been reported to expert multiple biological effects such as anti-inflammatory, anti-allergic, anti-viral and anti-cancer activities [25].

Thus, the results of this study confirmed the traditional uses, claiming that the plants viz., Anisolmeles malabarica and Clerodendrum serratum have potent antirheumatic and anti-pyretic properties. However, to know the exact mechanism of action of both these plants, further studies are being undertaken to isolate and characterize the specific chemical components that can be effectively used for the treatment of rheumatism.

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