ANTIOXIDANT POTENTIAL OF Sida retusa, Urena lobata AND Triumfetta rhomboidea

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ABSTRACT

The methanolic extract of Sida retusa Linn. (Malvaceae), Urena lobata Linn. (Malvaceae) and Triumfetta rhomboidea Jacq. (Teliaceae) roots were found to inhibit lipid peroxidation, scavenge hydroxyl and superoxide radicals in vitro. The quantity of S. retusa root extract required for 50% inhibition of lipid peroxidation, scavenging hydroxyl radical and superoxide radical was 1130.24 µg/ml respectively. IC 50 of root extract of U. lobata was 470.60 µg/ml, 1627.35µg/ml and 1109.24 µg/ml for superoxide radical scavenging, hydroxyl radical scavenging and lipid peroxidation respectively. T. rhomboidea root extract required for IC 50 was 336.65 µg/ml, 1346.03 µg/ml and 1004.22 µg/ml for superoxide scavenging, hydroxyl radical scavenging and lipid peroxidation respectively. The present investigation indicated that S. retusa, U. lobata and T. rhomboidea possessed significant antioxidant activity.

KEYWORDS

Sida retusa, Urena lobata, Triumfetta rhomboidea, antioxidant.

INTRODUCTION

Sida retusa Linn. (Bala) an Ayurvedic medicinal plants forms a major ingredient of the famous medicine Kshirabala used in the treatment of rheumatism. Urena lobata Linn. Is considered as a substitute of S. retusa1. Information collected from local informants revealed that Triumfetta rhomboidea Jacq. is an adulterant of S. retusa and it has shown antiulcer activity2. Since no experimental work has been carried out to verify the claims on the antioxidant activity of S. retusa root, this study was undertaken to evaluate the in vitro antioxidant activity of methanolic extract of S. retusa, U. lobata and T. rhomboidea roots.

Reactive oxygen species (ROS) such as superoxide (O₂⁻), hydroxyl radicals (OH⁻) and hydrogen peroxide (H₂O₂⁻) form an important factor in the etiology of several pathological conditions such as Alzheimer’s disease, Parkinson’s disease, arthritis, haemorrhoids, rheumatism, heart attack, AIDS, immune system and disorders, cataract, stroke, cancer stress, varicose veins, hepatitis, diabetes and several degenerative diseases.
including aging \textsuperscript{3,4,5,6}. ROS are degraded to non-reactive forms by enzymatic and non-enzymatic antioxidant defense mechanisms.

\textit{Sida retusa} Linn. (Bala) is a very important Ayurvedic medicinal plant, forms a chief ingredient of the famous medicine \textit{Kshirabala} used in the treatment of rheumatism. \textit{Urena lobata} Linn. is considered as a substitute of \textit{S.retusa} (Iyer and Kolammal 1993). Information collected from local informants revealed that \textit{Triumfetta rhomboidea} Jacq. is an adulterant of \textit{S.retusa} available literature, showed that no experimental work has been carried out to verify the claims on the antioxidant activity of \textit{S.retusa} root. Therefore, it was this study was undertaken to evaluate the \textit{in vitro} antioxidant activity of methanolic extract of \textit{S. retusa}, \textit{U.lobata} and \textit{T.rhomboidea} roots.

\section*{MATERIALS AND METHODS}

\subsection*{Plant material}

\textit{S.retusa}, \textit{U.lobata} and \textit{T.rhomboidea} were collected from Kolenchery during June-July and identified by Dr. Pradeep A. K., Herbarium Curator, Department of Botany, Calicut University, Kerala. Voucher specimens were kept in the Department of Botany herbarium at U.C. College, Aluva, Kerala.

\subsection*{Preparation plant extract}

The root system was separated, dried under shade, powdered and extracted with methanol using Sohxlet apparatus. The extract was evaporated to dryness, resuspended in methanol and used for testing \textit{in vitro} antioxidant activity.

\section*{Preparation of liver homogenate}

The liver of male Wistar rat was used for preparing liver homogenate. 25\% (w/v) liver homogenate was prepared in 30 nM KCl. The tissue ground well in a cold mortar and pestle, was kept the homogenate for 15 minutes under cold condition. The clear homogenate was collected from the top for studies.

\section*{Hydroxyl radical scavenging activity}

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe\textsuperscript{3+} / ascorbate /EDTA/H\textsubscript{2}O\textsubscript{2} system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS (thiobarbituric acid reactive substance) formation\textsuperscript{7}. The reaction mixture contained deoxyribose (2.8mM), FeCl\textsubscript{3} (0.1mM), EDTA (0.1mM), H\textsubscript{2}O\textsubscript{2} (1mM), Ascorbate (0.1 mM) KH\textsubscript{2}PO\textsubscript{4} – KOH buffer (20mM, pH7.4) and various concentrations of the drug (400-2000ug) in a final 1ml volume. The reaction mixture was incubated for 1 hour at 37\textdegree C. Deoxyribose degradation was measured as TBARS method\textsuperscript{8} and the percentage of inhibition was calculated.

\section*{Superoxide radicals scavenging activity}

Superoxide radical scavenging activity was determined by the nitroblue tetrazolium (NBT) reduction method\textsuperscript{9}. The reaction mixture contained EDTA (0.1mM) containing 0.0015\% NaCN, riboflavin (0.12mM), NBT (1.5mM), various concentrations of the extract (200-1000 ug) and phosphate buffer (pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated under an incandescent lamp for 15 minutes and the
optical density was measured at 530 nm before and after illumination. The percentage of inhibition of superoxide generation was evaluated by comparing for absorbance values of the control and experimental tubes.

**Inhibition of lipid peroxide formation**

The lipid peroxide formation was measured by measuring the colour of thiobarbituric and reactive substance (TBARS) formed at the end of the reaction. Malondialdehyde (MDA), which is formed, as the end product in lipid peroxidation react with thiobarbituric acid (TBA) to give TBARS, which is pink in colour, measured at 530 nm. The reaction mixture, which contained rat liver homogenate (0.1ml 25% (w/v) in Tris-HCl buffer (20mM, pH 7.0), KCl (150mM), ferrous ammonium sulphate (0.8mM), ascorbic acid (0.3mM) and various concentrations of the drug (400-2000ug) in a final volume of 0.5 ml, was incubated for 1 hour at 37°C. After the incubation period, 0.4 ml was removed and treated with 0.2 ml of 8% Sodium dodecyl sulphate (SDS), 1.5 ml of 8% thiobarbituric acid, and 1.5 ml 20% acetic acid (pH3.5). The total volume was made up to 4ml with distilled water and then kept in a waterbath at 95-100°C for 1 hour. After cooling, 1 ml of distilled water and 5 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture which was shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The absorbance of the organic layer was measured at 560 nm after centrifugation. The percentage inhibition was determined by comparing the results of the drug treated and untreated samples.

**Statistical analysis**

All the results were expressed as mean ±SE of 4 replicates. Probit analysis was done to find the IC 50 of each test.

**RESULTS AND DISCUSSION**

**Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity calculated by hydroxyl radicals generated by the Fe³⁺ / ascorbate / EDTA / H₂O₂ system was found inhibited by the addition of the root extract of *S.retusa, U.lobata* and *T.rhomboidea* root. The concentrations of root extract needed for 50% inhibition were 1763.22 ug/ml, 1627.35 ug/ml and 1346.03 ug/ml for *S.retusa, U.lobata* and *T.rhomboidea* respectively (Table 1).

**Superoxide scavenging activity**

The methanolic extract of the root *S.retusa, U.lobata* and *T.rhomboidea* was found to scavenge the superoxide radical generated by photoreduction of riboflavin. The concentration required for 50% inhibition of scavenging of superoxide radical was found to be 71.29 ug/ml, 470.60 ug/ml and 336.65 ug/ml for *S.retusa, U.lobata* and *T.rhomboidea* respectively (Table 1).

**Inhibition of lipid peroxidation**

The generation of lipid peroxides by Fe²⁺/ascorbate in rat liver homogenate was found inhibited by the addition of the root extract of *S.retusa, U.lobata* and *T.rhomboidea*. The concentration needed for 50% inhibition was 1130.24 ug/ml, 1109.24 ug/ml and 1004.22 ug/ml (Table 1) respectively.
Reactive oxygen species (ROS) can initiate a wide range of toxic oxidation reactions, and these toxicities are likely to play a role in the pathophysiology of a number of diseases. There is a large amount of evidence to show that the production of ROS such as $O_2^-$, $H_2O_2^-$ and OH- occurs at the site of inflammation and that they contribute to tissue damage$^{12}$. Roots of $S$. retusa are used for treatment of rheumatism$^{13}$. $U$. lobata has antibacterial activity$^{14}$ and $T$. rhomboidea has antiulcer activity$^{16}$. Inflammation mainly caused by generation of free radical hence, the administration of antioxidants may have a protective role in these conditions. The findings of present study gave emphasis to this factor. 71.29-ug/ml extract of $S$. retusa root provide 50% inhibition of superoxide radical scavenging activity. 1763.22 ug/ml and 1130.24 ug/ml of root extract $S$. retusa is needed for 50% inhibition of hydroxyl scavenging activity and lipid peroxidation respectively. 71.29-ug/ml extract of $S$. retusa root provide 50% inhibition of superoxide radical scavenging activity. 1763.22 ug/ml and 1130.24 ug/ml of root extract $S$. retusa is needed for 50% inhibition of hydroxyl scavenging activity and lipid peroxidation respectively. 71.29-ug/ml extract of $S$. retusa root provide 50% inhibition of superoxide radical scavenging activity. 1763.22 ug/ml and 1130.24 ug/ml of root extract $S$. retusa is needed for 50% inhibition of hydroxyl scavenging activity and lipid peroxidation respectively (Table 1). The present investigation indicates that $S$. retusa, $U$. lobata and $T$. rhomboidea possessed significant antioxidant activity.

The present investigation indicates that $S$. retusa possessed significant antioxidant activity. The significant antioxidant activity of this plant might be responsible for its treatment towards rheumatism. The antioxidant property of the three plants studied revealed that the original, substitute and adulterant have medicinal properties and the concentrations required for IC 50 varied from taxa to taxa. Phytosterols, alkaloids and fatty acids are present in $S$. retusa$^{15}$ that may be responsible for its antioxidant property. Such chemical constituents may be present in substitute and adulterant may also have antioxidant property. The chemical constituents responsible for antioxidant property are present in all the three plants in varying concentrations.

Table 1. Effect of the methanolic extract of plant materials on oxygen derived free radical generation.

| Test material  | Superoxide radical (ug/ml) | Hydroxyl radical (ug/ml) | Lipid peroxidation (ug/ml) |
|---------------|-----------------------------|--------------------------|---------------------------|
| $S$. retusa   | 71.29±13.61                 | 1763.22±211.43           | 1130.24±112.08            |
| $U$. lobata   | 470.60±17.17                | 1627.35±182.46           | 1109.24±141.39            |
| $T$. rhomboidea | 336.65±43.18              | 1346.03±116.76           | 1004.22±125.48            |

Values are mean ±SE(n=4)

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