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Research Article

ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF *PTEROSPERMUM RUBIGINOSUM* HEYNE EX WIGHT AND ARN AND *PTEROSPERMUM RETICULATUM* WIGHT AND ARN (STERCULIACEAE): AN *IN VITRO* COMPARATIVE STUDY

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ABSTRACT

Objectives: Plants from the family Sterculiaceae are used as folk medicine for treating various diseases in India. This study aims to determine the antioxidant and anti-inflammatory properties of *Pterospermum rubiginosum* and *Pterospermum reticulatum* of the family Sterculiaceae. The barks of *P. rubiginosum* and *P. reticulatum* are used in traditional medicine especially in the treatment of wounds, sprains, bone fracture, etc. This study, we compare the antioxidant and anti-inflammatory potentials of the stem bark of these two plants.

Methods: The free radical scavenging assays such as 2,2–diphenyl,1–picrylhydrazyl (DPPH), 2,2'–azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) (ABTS), hydroxyl radical, nitric oxide radical, phosphormolybdenum assay, and reducing power assay are used for the measurement of antioxidant potentials. The *in vitro* anti-inflammatory activities of the extracts are evaluated by means of lipoxygenase (LOX) and protease inhibition.

Results: Both *P. rubiginosum* and *P. reticulatum* scavenge DPPH (70.10% and 91.02%), ABTS (94.48 and 98.19%), hydroxy (76.02 and 87.67%), and nitric oxide (87.02 and 80.84%) radicals. Phosphomolybdenum assay and reducing power assay, used for the measurement of antioxidant potentials also showed good results. Regarding the anti-inflammatory potential, the methanolic extract of the plants shows anti-protease activity (51.29 and 64.93%) and anti-LOX activity (56%) while *P. rubiginosum* does not exhibit anti-LOX activity.

Conclusion: The above results demonstrate that the plants *P. rubiginosum* and *P. reticulatum* are rich source of antioxidant and anti-inflammatory compounds and it is the first report on theantioxidant and anti-inflammatory properties of the barks of these plants.

Keywords: Antioxidants, Free radicals, Inflammation, Oxidative stress, *Pterospermum reticulatum, Pterospermum rubiginosum.*

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INTRODUCTION

The production of free radicals such as superoxide, peroxide, and nitric oxide harms macromolecules such as proteins, lipids, and nucleic acids in human cells. This will lead to diseases such as cancer, acute, and chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis, and aging [1-6]. The antioxidant enzymes and antioxidant compounds such as ascorbic acid, tocopherol, and glutathione prevent the cells from the possible harms that free radicals can cause or they act as "free radical scavengers." Inflammation is a response of body tissues to the harmful effects caused by physical injury, chemical substances, and certain microbial agents. It is initiated by the release of inflammatory mediators from injured tissues and migrating cells [7]. This problematic condition attracted the researchers to study the effect of antioxidant activity in inflammatory diseases. In spite of the discovery of several novel agents, the search for better anti-inflammatory drugs still continues due to their side effects, especially during the prolonged course. In this context, various potent drugs of plant origin are used widely in Indian traditional system of medicine.

Pterospermum rubiginosum is a tree which belongs to the family Sterculiaceae in evergreen forests of Indian states such as Assam, Karnataka, Tamil Nadu, and Kerala at an altitude of up to 1000 m. Although the bark of *P. rubiginosum* has been reported to be a traditional medicine in India, the anti-inflammatory and antioxidant effect of it remains unexplored. *Pterospermum reticulatum* is also a medicinally important tree belonging to the same family. This tree is found in the evergreen forests of Western Ghats of India at low altitudes, and the stem bark of this plant had been used in India to treat ulcers, wounds, and inflammation. However, there are no reliable scientific reports available regarding the *in vitro* antioxidant and anti-inflammatory potentials of the barks of these two plants. The present work focuses on *in vitro* antioxidant and anti-inflammatory activity of methanolic extracts from the bark of *P. rubiginosum* and *P. reticulatum* considering the ethnomedical importance of these plants.

METHODS

Collection, authentication, and extraction of bark materials

The bark of *P. rubiginosum* and *P. reticulatum* was collected from Swaminathan Research Foundation and Tropical Botanical Garden Research Institute, Kerala, India. The bark material was identified and authenticated from the Department of Botany, Government Brennen College, Thalassery, under Kannur University. The specimen was preserved in the College Herbarium (Voucher.No.16315 and 16314). The barks of the *P. rubiginosum* and *P. reticulatum* (30 g each) were dried in the shade and then powdered using a mechanical grinder. The powdered bark materials were subjected to methanol (300 ml) extraction using Soxhlet apparatus. The solvent was completely removed using rotor evaporator and the extract (5.1 g and 3.9 g); thus, obtained was stored for further use.

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITIES

The antioxidant activity of the methanolic extracts from the bark of *P. rubiginosum* and *P. reticulatum* was evaluated and measured by the following methods such as 2, 2–diphenyl, 1–picrylhydrazyl (DPPH) assay [8], 2,2– azino– bis(3-ethylbenzothiozoline-6-sulfonic acid) (ABTS) assay [9], nitric oxide radical scavenging assay [10], and hydroxyl radical scavenging assay [11]. The IC_{50} of the bark extracts were calculated. Phosphomolybdenum assay [12] and reducing power assay [13] were also determined. The assays were done at a concentration ranging from 200 µg/ml to1000 µg/ml for both the sample.

Evaluation of anti-inflammatory activities

Antiprotease activity

The enzymatic assay for trypsin inhibition was carried out using the spectrophotometric assay by Sigma-Aldrich with certain modifications. In the experiment, 200 µl of trypsin is added with 200 µl of the sample solution and incubated for 10 min. The reaction is then initiated by the addition of 3 ml of substrate N-benzoyl-L- arginine ethyl ester and the absorbance is measured at 253 nm for 10 min using ultraviolet (UV) visible spectrophotometer. Phenyl methyl sulfonyl fluoride (PMSF), a known trypsin inhibitor is used as positive control. The inhibition of trypsin is calculated by following the equation:

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

Where A is the change in absorbance without test sample and B is the change in absorbance with the test solution.

***In vitro* lipoxygenase (LOX) inhibition assay**

The inhibition of LOX is determined by spectrophotometric assay using Soybean LOX-13. The substrate linoleic acid is used for the experiment. The conversion of linoleic acid to 13-hydroperoxy linoleic acid is followed spectrophotometrically by the appearance of a conjugated diene at 234 nm on a UV/visible spectrophotometer with some modifications, and the reaction rate is determined [14]. The mixture which contains 50 µl of LOX enzyme added with 50 µl of test solution of suitable concentration and is incubated for 1 min. Different volumes of the buffer are added to this mixture, and the reaction is initiated by adding 360 µl substrate and recorded at 234 nm for 5 min using UV-visible spectrophotometer. Vanillin, a known inhibitor of LOX, is used as a standard.

The inhibition of LOX, calculated by following the equation:

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

Where A is the change in absorbance without test sample and B is the change in absorbance with the test solution.

RESULTS AND DISCUSSION

This study puts forward the use of natural antioxidants in the treatment of diseases caused by oxidative stress and inflammatory conditions. Plants are the important source of natural antioxidants. The usefulness of natural antioxidants in preventing the harmful consequences of oxidative stress and inflammatory conditions has created an increasing interest in the protective biochemical functions of natural antioxidants contained in medicinal plants [15]. The two medicinal plants *P. rubiginosum* and *P. reticulatum* have not been reported yet for their activity against oxidative stress and inflammation. The methanolic extract yield of *P. rubiginosum* and *P. reticulatum* taken for the analysis is 17% and 13%, respectively.

***In vitro* free radical scavenging effect of the extract**

DPPH method

The percentage of scavenging was 7.71±0.73 at a minimum concentration of 200 µg/ml and 70.10±1.82 at a maximum concentration of 1000 µg/ml for *P. rubiginosum*. The percentage of scavenging activity of *P. reticulatum* showed inhibition of 50±0.19 at an initial concentration of 200 µg/ml and 91% at a concentration of 1000 µg/ml (Fig. 1). Although both the plants exhibited a significant radical scavenging effect on the DPPH radicals, the effect was higher for *P. reticulatum* when compared to *P. rubiginosum*. The IC_{50} of *P. rubiginosum* was found to be 0.714 mg/ml where as the *P. reticulatum* shows an IC_{50} of 0.15 mg/ml. Similarly, some Algerian medicinal plants such as *Olea europaea* and *Ziziphus lotus* scavenge DPPH radicals with increased concentrations [16].

ABTS radical scavenging assay method

The percentage of scavenging in *P. rubiginosum* ranged from 54.66±1.54 with a minimum concentration range of 200 µg/ml and 94.48±0.83 at a maximum concentration of 1000 µg/ml. *P. reticulatum* showed inhibition of 98% at a high concentration of 1000 µg/ml (Fig. 2). It proves that the plant extract is useful for treating free radical-related inflammatory disorders. The IC_{50} of *P. rubiginosum* was found to be 0.182 mg/ml whereas the *P. reticulatum* showed an IC_{50} of 0.166 mg ml. Similarly, the plant *Pedilanthus tithymaloides* under Euphorbiaceae shows high inhibition of 98.56% on stable ABTS radicals [17].

Hydroxyl radical scavenging assay method

The methanolic extract of *P. rubiginosum* extract showed better scavenging activity for hydroxyl free radicals, and the percentage of scavenging ranges from 21.31±1.55 for a minimum concentration of 200 µg/ml to 76.02±1.87 at a maximum concentration range of 1000 µg/ml. In the case of *P. reticulatum*, the inhibition was found to be 87% for methanol extract of the bark at a high concentration of 1000 µg/ml (Fig. 3). The IC_{50} of *P. rubiginosum* was found to be 0.648 mg/ml whereas the *P. reticulatum* showed an IC_{50} of 0.512 mg/ml.

Nitric oxide scavenging assay method

A better scavenging activity for nitric oxide radicals was observed for *P. rubiginosum* at a range from 51.31±0.55 at a minimum concentration of 200 µg/ml to 87.02±2.59 at a maximum concentration of 1000 µg/ml. *P. reticulatum* showed 81% activity at a high concentration of 1000 µg/ml (Fig. 4). Nitric oxide is involved in oxidative stress and various inflammatory processes, and it acts as a potent inhibitor for several processes such as relaxation of smooth muscles, neuronal signaling, and aggregation of blood platelets [18]. Better nitric oxide scavenging activity shown by both *P. rubiginosum* and *P. reticulatum* is a good indication of its usefulness in the treatment of inflammation. The IC_{50} of *P. rubiginosum* was found to be 0.195 mg/ml whereas the *P. reticulatum* showed an IC_{50} of 0.2 mg/ml.

***In vitro* antioxidant activity of the extract**

Ferric reducing power assay

About 50 µg/ml equivalents of standard ascorbic acid were found to be equivalent to 200 µg/ml of the methanolic extract of *P. rubiginosum,* and

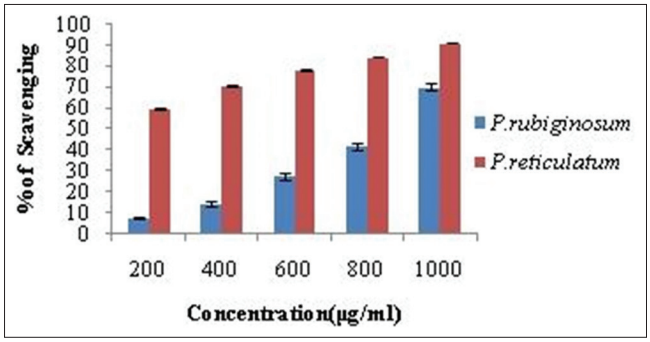

Fig. 1: Percentage scavenging (mean±standard deviation) by 2,2–diphenyl,1–picrylhydrazyl assay

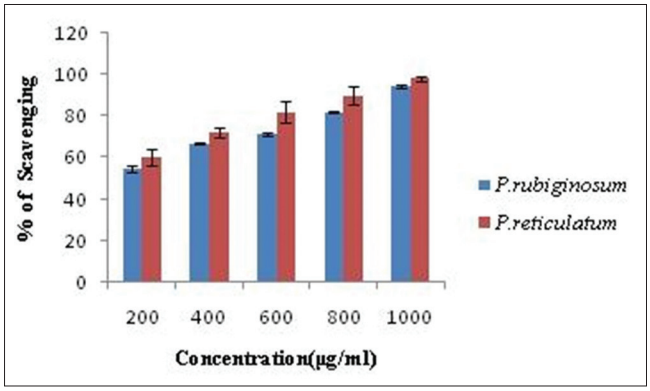

Fig. 2: Percentage scavenging (mean±standard deviation) by 2,2'–azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) assay

200 µg/ml of methanolic bark extract of *P. reticulatum* was found to be equivalent to 77 µg/ml of ascorbic acid (Fig. 5). As the optical density of these bark extracts is very high, its reductive ability increases. These plant extracts have natural antioxidants to donate electrons and to reduce Fe+3–Fe+2 ions. Hence, both the plants have better reducing power activity.

Phosphomolybdenum assay

Phosphomolybdenum assay was used for screening antioxidant activity of plant extracts. Here, 200 µg/ml of the *P. rubiginosum* was found to be equivalent to 128 µg/ml of standard ascorbic acid. However, in *P. reticulatum* 200 µg/ml of bark methanol extract was found to be equivalent to 100 µg/ml of ascorbic acid (Fig. 6). Increase in optical density indicates a higher antioxidant activity of the plant. The methanolic extracts of both the plants reduced Mo (VI) to Mo (V) in the presence of natural antioxidants, which are found in these medicinal plants. Thus, the activity increases with increase in the concentration of *P. rubiginosum* and *P. reticulatum.*

In vitro anti-inflammatory assays

Antiprotease activity

P. rubiginosum and *P. reticulatum* exhibited significant antiprotease activity at different concentrations. *P. rubiginosum* showed an inhibition of 51.29% at 500 µg/ml and *P. reticulatum* showed an inhibition of 64.93% at 500 µg/ml whereas PMSF, a standard protease inhibitor showed 55.19% of inhibition at 100 µg/ml (Fig. 7). Neutrophils contain proteases localized in lysosomes, and these proteases are involved in the development of tissue damage during chronic inflammatory conditions [19]. In this study, *P. rubiginosum* and *P. reticulatum* proved to be having efficient antiprotease activity, which may help in overcoming inflammatory conditions.

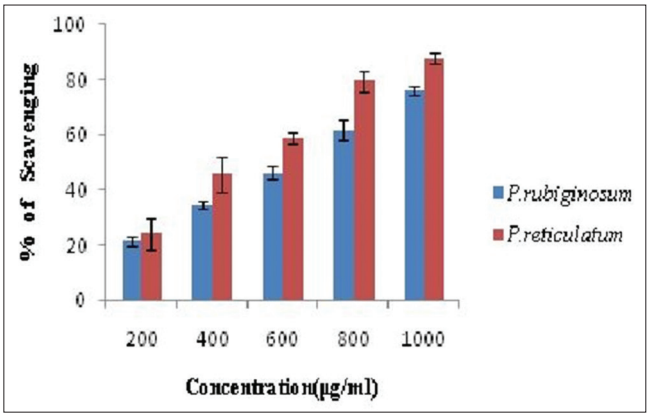

Fig. 3: Percentage scavenging (mean±standard deviation) by hydroxy radical scavenging assay

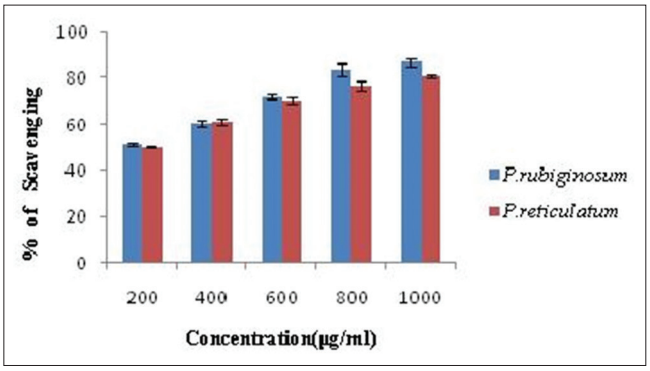

Fig. 4: Percentage scavenging (mean±standard deviation) by nitric oxide scavenging assay

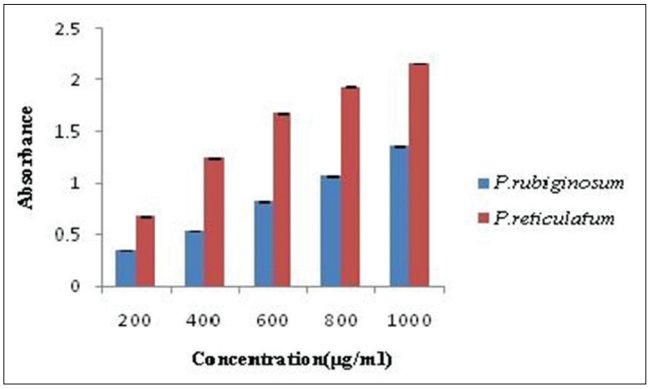

Fig. 5: Antioxidant activity by ferric reducing power assay

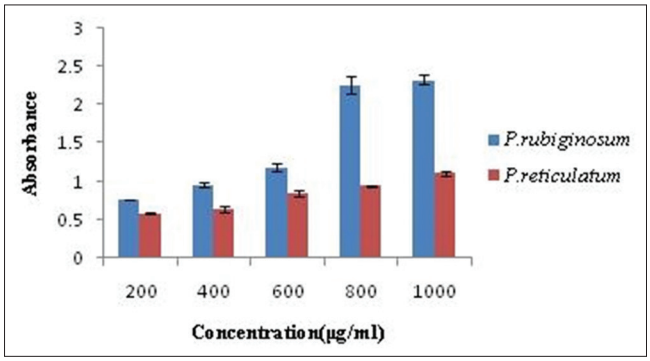

Fig. 6: Antioxidant activity by phosphomolybdenum assay

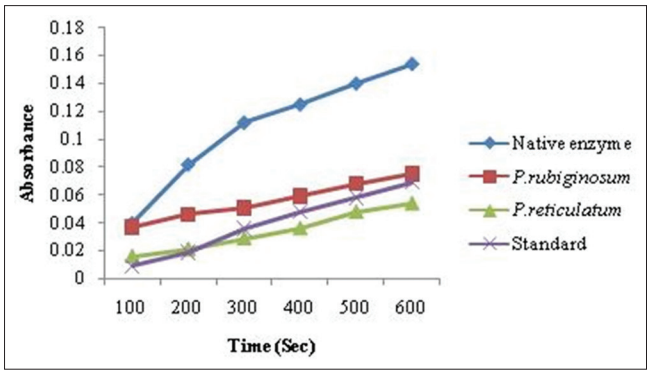

Fig. 7: Antiprotease activity of *Pterospermum rubiginosum* and *Pterospermum reticulatum*

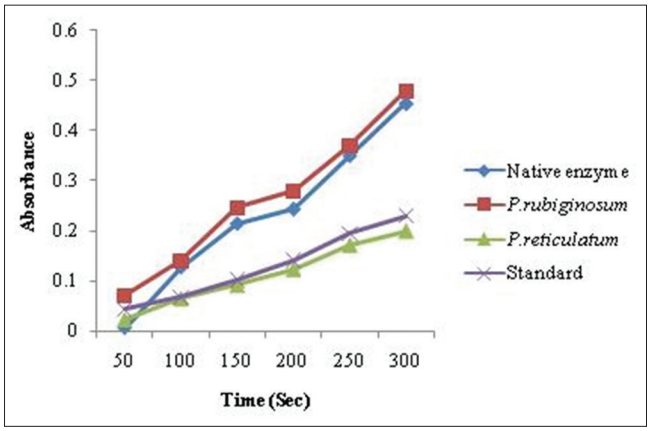

Fig. 8: Antilipoxygenase activity of *Pterospermum rubiginosum* and *Pterospermum reticulatum*

***In vitro* LOX inhibition assay**

Soybean 13-LOX was used for this study as it is found to be one of the most stable enzymes among LOXs. The methanolic bark extract of *P. reticulatum* showed 56% inhibition whereas *P. rubiginosum* showed no inhibition at a concentration of 500 µg/ml. The standard inhibitor vanillin shows inhibition of 49.33% at a concentration of 100 µg/ml (Fig. 8). In our findings, *P. reticulatum* acted as an inhibitor for LOX, which plays a key role in the prostaglandin biosynthesis, where the prostaglandins are the mediators of inflammation. Therefore, this activity can be made useful against various inflammatory disorders in the human system.

CONCLUSION

This study shows that the plants *P. rubiginosum* and *P. reticulatum* are rich sources of antioxidants and anti-inflammatory compounds. It is the first report in which the antioxidant and anti-inflammatory properties of these plants have been investigated. These findings now need to be validated with animal models for better management of human diseases resulting from oxidative stress and inflammation. The efforts for purification and identification of the active compounds from both the plants are still in progress.

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AUTHORS' CONTRIBUTION

Jenson Jacob collected the data, conducted the experiment, and prepared the article. Dr. K. Sreejith, Professor, supervised the experiments and reviewed the article.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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