RESEARCH ARTICLE

Preliminary phytochemical analysis, antiradical and lipid peroxidation inhibitory activity of *Rubus steudneri* Schweinf. (Rosaceae)

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Abstract

Objectives: Free radical is a molecular species that contain an unpaired electron in an atomic orbital. Free radicals are highly reactive, capable of damaging biomolecules such as proteins, nucleic acid, and lipids, and cause oxidative damage. The present study was carried out to determine antiradical and lipid peroxidation inhibitory activity of leaf extract of *Rubus steudneri* Schweinf. belonging to the family Rosaceae. **Materials and Methods:** Extraction of powdered leaf material was carried out by maceration process. Leaf extract was subjected to preliminary phytochemical analysis by standard tests. Leaf extract was screened for antiradical (2,2-diphenyl-1-picrylhydrazyl [DPPH] and hydroxyl radical scavenging assay) and lipid peroxidation inhibition activity. Total phenolic and flavonoid content was estimated by Folin–Ciocalteu reagent method and aluminum chloride colorimetric estimation method, respectively. **Results:** Phytochemicals, namely, saponins, alkaloids, flavonoids, and tannins were detected in the leaf extract. The extract was shown to scavenge DPPH and hydroxyl radicals dose dependently with IC_{50} value 49.99 µg/ml and 15.19 µg/ml, respectively. Inhibition of lipid peroxidation by leaf extract was also dose dependent (IC_{50} value 27.72 µg/ml). The content of total phenolics and flavonoids was found to be 133.51 mg gallic acid equivalents/100 g of extract and 80.61 mg catechin equivalents/100 g of extract, respectively. **Conclusion:** The leaf extract of *R. steudneri* exhibited a marked concentration-dependent antiradical and lipid peroxidation inhibitory activity which can be attributed to the phenolic and flavonoid content. The plant can be used for the prevention and management of oxidative damage induced by free radicals.

Key words: Free radical, lipid peroxidation, maceration, phytochemical, *Rubus steudneri*

INTRODUCTION

Free radical is any molecular species having an unpaired electron in its outer orbital. Many of the free radicals are highly unstable and reactive. Reactive oxygen species (such as superoxide radical, hydroxyl radical, peroxyl radical, singlet oxygen, and hydrogen peroxide) and other reactive species (such as nitric oxide, peroxynitrite, peroxynitrous acid, and nitrogen dioxide) are constantly produced in the body during metabolism and under certain other circumstances. These radicals are implicated in oxidative damage which leads to several pathophysiological conditions such as aging, cancer, cardiovascular diseases, diabetes, cataracts, and neurodegenerative disorders. To counteract the damaging effects of free radicals, cells possess an antioxidant defense system which involves enzymatic (superoxide dismutase, peroxidase, and catalase) and non-enzymatic (such as ascorbic acid, Vitamin E, glutathione, and carotenoids) mechanisms. There exists a balance between free radical generation and antioxidant defense in normal health. Conditions such as overproduction of radicals and a deficit in antioxidant defense of the cell disturb the equilibrium, resulting in oxidative damage. Consumption of antioxidants from exogenous sources such as diet is much needed to prevent and control oxidative damage. An immense interested on antioxidant potential of plants is

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triggered due to suspected carcinogenic or toxic effect of synthetic antioxidants. Phenolic and flavonoid compounds are shown to be potent antioxidant phytochemicals in plants. Studies have shown that extracts and purified metabolites (in particular, phenolic and flavonoids) of plants exhibit antioxidant activity.[1-11]

Rubus steudneri Schweinf. (commonly called gora; [Figure 1]) belonging to the family Rosaceae is a scandent shrub with deeply furrowed stems that are grayish-tomentose and covered with stellate hairs or sometimes with prickles. Leaves are trifoliolate, doubly serrate, glabrate above, and densely whitish-tomentose below. Inflorescence is a panicle which is terminal, much-branched, and the branches are glandular-tomentose. Petals are purplish in color, obovate-oblong, and twice as long as the calyx. Fruits are edible. The plant is used traditionally as food as well as medicine. The decoction prepared from the roots is taken as a remedy for indigestion, gastritis, and diarrhea. The plant is also used to treat diabetes mellitus.[12,13] Various parts of the plant, namely, leaf, stem, and fruit are eaten by gorillas in Bwindi Impenetrable National Park, Uganda.[14]

The leaves of *R. steudneri* are used traditionally to treat rheumatism in Bale Mountains National Park, Southeastern Ethiopia.[15] The roots of *R. steudneri* are used for the treatment of stomachache with diarrhea by Meinit ethnic group of Ethiopia.[16] In Kabira subcounty, Rakai District, the decoction made from the roots of *R. steudneri* is used to relieve a cough in cattle.[17] In Wonago Woreda, Ethiopia, the plant is used for food and ornamental purposes.[18] The fruit is consumed as edible food in the different regions of coffee forests of Ethiopia.[19] In Ada's district, Ethiopia, *R. steudneri* is used to treat both human and veterinary ailments.[20] Stem of *R. steudneri* is used as fence and for house construction in Southwest Ethiopia.[21] In Aris zone of Central Ethiopia, the plant is grown for edible fruits that are eaten.[22] In Kabira subcounty, Rakai district, Southwestern region of Uganda, the decoction prepared from roots is administered to cattle as a remedy for cough.[17] The fruit of the plant is consumed as an edible in Berehet District, Ethiopia, in the form of an additional food.[23] *R. steudneri* is used as a wild edible plant in Burji district, Ethiopia.[24] The Gumuz community in western Ethiopia uses fruit of the plant for edible purpose.[25] Studies have shown that the plant exhibits antimicrobial,[12,17] antioxidant,[26,27] and antiproliferative activities.[27] The objective of the present study was to screen the phytochemicals and to evaluate antiradical and lipid peroxidation inhibitory activity of *R. steudneri* leaf extract.

MATERIALS AND METHODS

Collection and Extraction

The plant *R. steudneri* was collected at Nekemte, located in the East Welega Zone of the Oromia Region, Ethiopia. The plant was authenticated based on its characteristics. The leaves were separated, washed well under running water, and dried under shade. Extraction was carried out by maceration process as described by Raghavendra *et al*.[28] with slight modification. 10 g of shade-dried leaf material was added to 100 ml of double-distilled water taken in a stoppered container. The container was left for about 72 h with occasional stirrings followed by filtering the content through muslin cloth. The filtrate was further filtered through Whatman No. 1 filter paper and evaporated to dryness.

Phytochemical Analysis of Leaf Extract

The concentrated methanolic leaf extract was subjected for preliminary phytochemical analysis by standard phytochemical tests to screen for the presence of phytochemicals, namely, alkaloids, flavonoids, saponins, glycosides, tannins, triterpenoids, and phytosterols.[29-31]

Preparation of Leaf Extract

The crude leaf extract was dissolved in methanol. Different extract concentrations ranging from 6.25 to 200 µg/ml of methanol were prepared by diluting the extract in methanol. These extract concentrations were subjected for antiradical and lipid peroxidation inhibitory activities.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The protocol of Chandrashekar *et al*.[32] was employed to evaluate DPPH radical scavenging potential of leaf extract of *R. steudneri*. The absorbance of reaction mixture was read at 517 nm spectrophotometrically. Ascorbic acid was used as reference standard. The scavenging of DPPH radicals by leaf extract (%) was determined using the formula:

Scavenging of DPPH radicals (%) = (Ac – At/Ac) × 100, where "Ac" and "At" refer to absorbance of DPPH control and absorbance of DPPH in the presence of extract/standard. IC_{50} value (the concentration of extract/standard required to scavenge 50% of radicals) was calculated.

Hydroxyl Radical Scavenging Assay

The method described by Jiang *et al*.[33] was employed to determine hydroxyl radical scavenging activity of leaf extract of *R. steudneri* and ascorbic acid (reference standard). The absorbance of reaction mixture was measured spectrophotometrically 532at nm. The scavenging of hydroxyl radicals (%) was calculated using the following formula:

Scavenging of hydroxyl radicals (%) = 1-(absorbance of sample/absorbance of blank) × 100. IC_{50} value was calculated.

IC_{50} value denotes the concentration of leaf extract/ascorbic acid required to scavenge 50% of free radicals.

Lipid Peroxidation Inhibition Assay

With minor modifications, the protocol employed by Badmus *et al*.[34] was used to evaluate the extent of inhibition of lipid peroxidation by leaf extract by estimating the thiobarbituric acid reactive substances. The absorbance of reaction mixture was measured at 532 nm spectrophotometrically. Ascorbic acid was used as reference standard. The percentage of inhibition of lipid peroxidation was calculated using the following formula:

Inhibition of lipid peroxidation (%) = (Ac – At/Ac) × 100, where "Ac" and "At" represent to absorbance of control and absorbance of test, respectively. IC_{50} value was calculated. IC_{50} value depicts the concentration of leaf extract/ascorbic acid required to cause 50% inhibition of lipid peroxidation.

Total Phenolic Content

The Folin–Ciocalteu's reagent (FCR) method described by Raghavendra *et al*.[35] was carried out to estimate the content of total phenolics in the leaf extract of *R. steudneri*. Gallic acid was used as reference standard. The absorbance or reaction mixture was read at 750 nm spectrophotometrically. The total phenolic content was expressed as mg gallic acid equivalents/100 g of dry extract.

Total Flavonoid Content

The aluminum chloride colorimetric estimation method described by Raghavendra *et al*.[35] was performed to estimate the content of total flavonoids in the leaf extract of *R. steudneri*. Catechin was used as reference standard. The absorbance of reaction mixture was measured at 510 nm. The flavonoid content of leaf extract was expressed as mg catechin equivalents/100 g of dry extract.

Statistical Analysis

All experiments were done in triplicates ($n = 3$) and the results are represented as mean ± standard deviation. IC_{50} value was calculated by linear regression analysis using Origin (Data Analysis and Graphing) Software version 7.0 for windows.

RESULTS AND DISCUSSIONS

Phytochemicals Detected in Leaf Extract of *R. steudneri*

Plants produce a range of primary and secondary metabolites. Therapeutic role of plants is ascribed to the presence of secondary metabolites in them. Among various such metabolites, compounds such as polyphenolic compounds, alkaloids, and terpenoids are major compounds. Studies have shown the potential of purified metabolites of plant origin to exhibit various pharmacological activities such as antimicrobial, antioxidant, and anti-inflammatory activity. Hence, it becomes important to screen extracts for the detection of phytochemicals.[29,30,36-41] In the present study, we screened methanolic leaf extract of *R. steudneri* for phytochemicals, and the result of preliminary phytochemical analysis of leaf extract is shown in Table 1. In the leaf extract, all phytochemicals except glycosides, phytosterols, and triterpenoids were presented.

Scavenging of DPPH Radicals by Leaf Extract of *R. steudneri*

DPPH is a commercially available, nitrogen-centered, stable, organic free radical that shows an absorption maximum at 515–520 nm in alcoholic solution. The method involving scavenging of DPPH radicals is one of the most extensively used *in vitro* antiradical assays. The method is simple, cheap, and the results are reproducible. Substances (i.e., antioxidants) having the potential to donate hydrogen cause bleaching of the purple-colored DPPH radicals and yellow-colored non-radical DPPHH. This method is widely used to evaluate antiradical activity of several samples including plant extracts and pure compounds.[6,42-46] In the present study, we evaluated the potential of leaf extract of *R. steudneri* to scavenge DPPH radicals, and the result is shown in Figure 2. The leaf extract displayed marked dose-dependent scavenging of DPPH radicals with IC_{50} value 49.99 µg/ml. A scavenging activity of 50% and higher was observed at concentration, namely, 50 and 12.5 µg/ml of leaf extract and ascorbic acid, respectively. At 200 µg/ml, extract and ascorbic acid exhibited a scavenging activity of 91.24% and 95.41%, respectively. Scavenging activity of ascorbic acid (IC_{50} value 16.80 µg/ml) was higher than that of leaf extract. In an earlier study, Tadesse *et al*.[26] evaluated DPPH scavenging potential of solvent extracts of *R. stuedneri* leaves and it was observed that 80% methanol extract exhibited marked scavenging activity followed by acetone and methanol extracts. In another study, Tauchen *et al*.[27] revealed scavenging of DPPH radicals by 80% ethanol extract of roots and leaves and twigs of *R. steudneri*.

Scavenging of Hydroxyl Radicals by Leaf Extract of *R. steudneri*

Hydroxyl radicals are one among the most highly reactive oxygen species generated in the body and are known to cause severe damage than most other free radicals. Hydroxyl radical is the neutral form of hydroxide ion, short-lived and attacks various biomolecules non-specifically. It can react with molecules at the site of its formation. These radicals are also considered as rapid initiators of peroxidation of lipids. Hydroxyl radicals are produced by Fenton reaction in which

H_2O_2 reacts with metal ions. Removal of these hydroxyl radicals by scavenging activity is very important antioxidant activity. Studies have shown the potential of extracts of plants to scavenge hydroxyl radicals.[33,34,45,47-52] In the present study, the leaf extract of *R. steudneri* was effective in scavenging hydroxyl radicals in a dose-dependent manner [Figure 3]. An inhibitory activity of 50% and higher was observed at 12.5 and 25 µg/ml concentration of leaf extract and ascorbic acid, respectively. Leaf extract was found to scavenge hydroxyl radicals more efficiently with IC_{50} value 15.19 µg/ml when compared to ascorbic acid (IC_{50} value 31.83 µg/ml).

Figure 1: *Rubus steudneri* (Photograph by Raghavendra H.L)

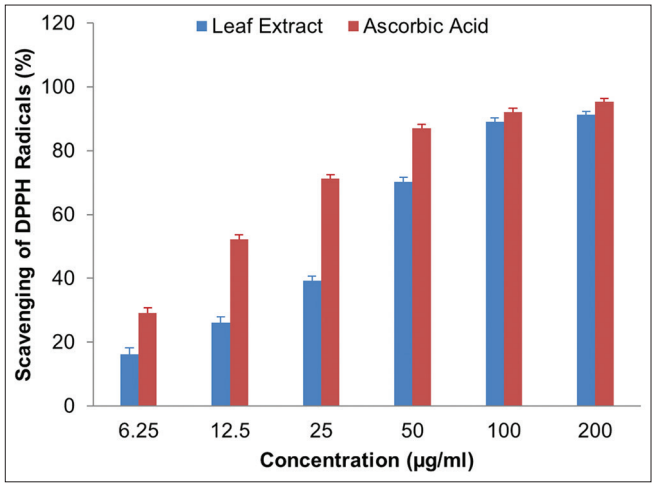

Figure 2: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of the leaf extract and ascorbic acid

Table 1: Phytochemicals detected in *R. steudneri* leaf extract

Phytochemicals	Leaf extract
Glycosides	–
Saponins	+
Alkaloids	+
Flavonoids	+
Phytosterols	–
Triterpenoids	–
Tannins	+

+: Detected; –: Not detected. *R. steudneri: Rubus steudneri*

Inhibition of Lipid Peroxidation by Leaf Extract of *R. steudneri*

The process of lipid peroxidation occurs through free radical chain reactions and results in damage to biological membrane. Damage caused to membrane leads to loss of membrane functioning such as decreased fluidity of membrane, inactivation of membrane-bound enzymes, and receptors. The reaction between free radicals and lipids results in the formation of lipid radicals which on reacting with molecular oxygen forms lipid peroxyl radicals. These radicals further propagate peroxidation process by extracting hydrogen atoms from other lipid molecules. Antioxidant species that donate hydrogen can break this propagation process and thereby inhibit peroxidation of lipids. It is shown that plant extracts possess lipid peroxidation inhibitory activity.[34,49,51,53,54] The result of lipid peroxidation inhibitory activity of leaf extract of *R. steudneri* is shown in Figure 4. The leaf extract as well

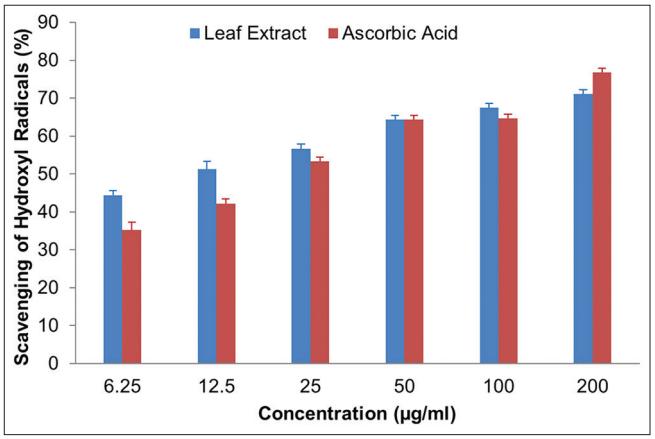

Figure 3: Hydroxyl radical scavenging activity of the leaf extract and ascorbic acid

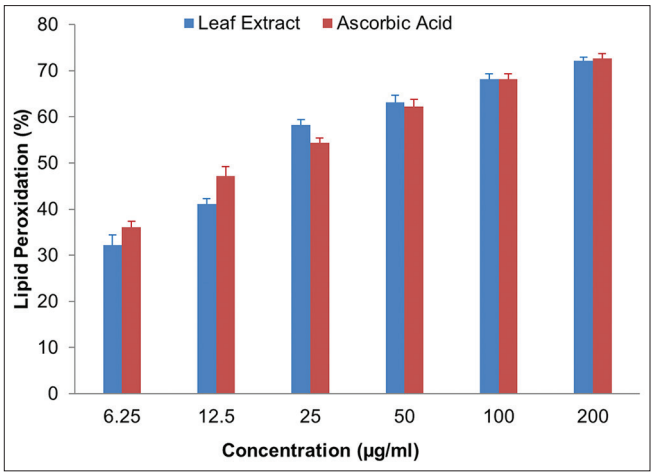

Figure 4: Inhibition of lipid peroxidation by leaf extract and ascorbic acid

as ascorbic acid exhibited marked concentration-dependent inhibition of lipid peroxidation. The activity observed was higher in case of ascorbic acid (IC_{50} value 19.99 µg/ml) when compared to leaf extract (IC_{50} value 27.72 µg/ml).

Total Phenolic and Flavonoid Content of Leaf Extract of *R. steudneri*

Polyphenolic compounds including flavonoids are widely distributed in various parts of the plants such as leaves, roots, seeds, and flowers. These compounds exhibit antioxidant activity by inactivating free radicals, chelating pro-oxidative metals, and quenching singlet oxygen. Many studies have shown a direct correlation between the phenolic and flavonoid content and antioxidant activity. Consumption of plants rich in polyphenol compounds can offer significant health benefit in terms of antioxidant potential.[54-58] FCR method and aluminum chloride colorimetric estimation method are widely used to estimate the content of total phenolics and flavonoids, respectively, in various kinds of samples including plant extracts.[54,56,57,59,60] The total phenolic content of leaf extract of *R. steudneri* was found to be 133.51 mg gallic acid equivalents/100 g of dry extract, while the total flavonoid content was 80.61 mg catechin equivalents/100 g of dry extract.

CONCLUSION

Free radicals are produced in the body and are known to cause deleterious effects. In the present study, the leaf extract of *R. steudneri* is shown to display marked antiradical and lipid peroxidation inhibitory activity. The observed antiradical and lipid peroxidation inhibitory activity of the plant could be ascribed to the presence of polyphenolic compounds. The lipid peroxidation inhibition activity observed could be ascribed to the radical scavenging potential of leaf extract. The plant is shown to be a good source of phytochemicals, and the results highlight the possibilities for the development of nutraceutical and food applications of the plant to offer health benefits. In suitable form, the plant can be used to prevent and manage oxidative damage caused by free radicals.

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