Research Article

Phytochemical screening and *in vitro* antioxidant activities of *Senna singueana* leaves

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ABSTRACT

Objective: *Senna singueana* deciduous shrub that belongs to the Fabaceae family occurs throughout mainland tropical Africa commonly called as scrambled egg, conventionally, in African contents, it has many medicinal uses to cure different types of disease such as anticancer, antimalarial, bilharzia, fever, and wounds caused by leprosy and syphilis. The objective of the study was to perform the qualitative phytochemical analysis and *in vitro* antioxidant activities of *S. singueana* leaves. **Materials and Methods:** The present study was to evaluate the qualitative phytochemical analysis and *in vitro* antioxidants activities of ethanolic extract of *S. singueana* leaves by 1,1-diphenyl-2-picrylhydrazyl, reducing power assay, hydroxyl radical, nitric oxide, and superoxide radical scavenging activities using standard methods.. **Results:** From the phytochemical analysis of different organic extracts, the ethanolic extract contained maximum number of secondary metabolites such as flavonoids, tannins and phenolic compounds, and terpenoid, and further, the *in vitro* antioxidant activity of ethanolic extract of *S. singueana* showed significant scavenging activity on increasing order of OH>DPPH>NO>O_2 compared to that of ascorbic acid (standard). **Conclusion:** Based on the present results, the ethanolic leaf extract of *S. singueana* contained flavonoids and phenols, thereby presented significant antioxidant activity, it can be used as a potential source of desired bioactive natural antioxidants for the development of therapeutic antioxidant drugs.

KEY WORDS: 2,2-diphenyl-1-picrylhydrazyl, Antioxidants, Ethanolic extract, Phytochemical analysis, *Senna singueana*

INTRODUCTION

Plant foods are not only a main source of nutrients but also they are rich in physiologically bioactive phytochemicals. Intake of fruit and vegetables is associated with a decreased risk of oxidative stress-related diseases. High amounts of free radical molecules cause oxidative stress in cells which result in damaging essential macromolecules including DNA, lipids, and proteins. Reactive oxygen and nitrogen species play a key role in the development of cancer, Parkinson's disease, atherosclerosis, aging, immunosuppression, ischemic heart disease, and diabetes.[1] It is usually documented that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation, and therefore, help in prevent oxidative stress-related diseases such as cancer and cardiovascular diseases.[2] Therefore, antioxidant functions of phytonutrients have been systematically investigated in the past few years in relation to their crucial effect in the pathophysiology associated with oxidative stress-related diseases. It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation, and therefore, help prevent cancer or heart diseases. A recent study has shown that *Senna singueana* root bark extracts have shown significant analgesic, antimicrobial, anthelminthic, and antiplasmodial activity.[3]

The objective of present study was to screen the phytoconstituents of different solvent extracts of *S. singueana* leaves and evaluate the *in vitro* antioxidant potential of ethanolic extract.

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MATERIALS AND METHODS

Plant Material Collection and Preparation

The leaves of *S. singueana* were collected from Axum Central Zone of Tigray Region, Ethiopia, in February 2017. The plant material was authenticated and

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specimen herbarium was deposited at Addis Ababa University, Biology Department, National herbarium of Ethiopia, and it was given the voucher specimen number of DG 001. The leaves were washed with tap water to remove dirts and impurities. Shade-dried at 25°C for 10 days and coarse powder.

Preparations of the Extracts

The leaves of *S. singueana* was dried at 25°C for 10 days in the absence of sunlight and powdered was using a mixer and stored in an airtight container. The powdered medicinal plant material was taken and subjected to serial exhaustive solvent extraction, during extraction solvents were diffuse into the plant material and solubilize the phytocompounds with similar polarity. For qualitative determination, the extracts were placed in pre-balanced flasks before drying. The remaining plant parts residues were extracted with other solvents sequentially.

Percentage Yield and Phytochemical Screening

The qualitative preliminary phytochemical analysis was carried out for petroleum ether, chloroform, ethyl acetate, and ethanol soluble fractions and carry out by the following methods as per the standard methods.[4,5]

Quantitative *In Vitro* Antioxidant Scavenging Assays

The ethanolic leaf extract of *S. singueana* was analyzed for *in vitro* antioxidant scavenging assays. The antioxidants activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH)[6] reducing power,[7] hydroxyl radical,[8] nitric oxide (NO) radical,[9] and superoxide radical scavenging assay/.[10] All the analysis was done in triplicates, and average values were taken.

DPPH radical scavenging activity

Various concentrations of ethanolic extract of (4.0 ml) *S. singueana* were mixed with 1.0 ml of ethanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.1 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as a control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\% \text{ decolorization} = [1-(ABS_{sample}/ABS_{control}]\times 100$$

Reducing power activity

The reaction mixture contained 2.5 ml of various concentrations of ethanolic extract, 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixtures were incubated at 50°C for 20 min, and were terminated by the addition of 2.5 ml of 10 % (w/v) of trichloroacetic acid followed by centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increased absorbance indicates increased reducing power of the ethanolic plant extract.

OH− radical scavenging assay

The reaction mixture (3.0 ml) contained 1.0 ml $FeSO_{4(}$1.5 mM), 0.7 ml hydrogen peroxide (6 mM), 0.3 ml sodium salicylate (20 mM), and varying concentrations of the ethanolic extract, allowed incubation for 1 h at 37°C, and the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as:

$$\text{Scavenging activity } (\%) = [1-(A_1-A_2)/A_0]\times 100$$

Where A_0 was the absorbance of the control (without extract), A_1 was the absorbance in the presence of the extract, and A_2 was the absorbance without sodium salicylate.

NO scavenging activity

Various concentration of the extract was mixed with 2.5 ml of sodium nitroprusside and made up to 3.0 ml with phosphate-buffered saline. Then, the mixture was incubated for 15 min at 25°C. After incubation, 0.5 ml of the reaction mixture was removed, and 0.5 ml of Griess reagent was added. Then, the absorbance was measured at 546 nm.

The percentage inhibition was calculated by comparing the results of the test with those of controls not treated with the extract, as per the following formula:

$$\% \text{ scavenging of Superoxide} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide radical activity

The assay tubes containing 20 µl of methanolic extract, 0.2 ml of ethylenediaminetetraacetic acid, 0.1 ml of nitro blue tetrazolium, 0.05 ml of riboflavin, and 2.25 ml of phosphate buffer and control tubes were set up without the extract. Similarly, the activity of the standard ascorbic acid was also carried out, allowed for incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank. A 560 nm was measured again, and the difference in optical density was taken as the quantum of superoxide production. The percentage inhibition was calculated by comparing with the optical density of the control tubes.

$$\% \text{ scavenging of Superoxide} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Statistical Analysis

The data for antioxidant activities were expressed as the average of three measurements, and all the

remaining data were expressed as mean ± standard deviations of triplicates using MS Excel 2015. The simplest estimate of inhibitory concentration (IC_{50}) was to plot x-y and fit the data with a straight line (linear regression). IC_{50} value is then estimated using the following formula:

$$y = a * x + b$$

$$IC_{50} = \frac{(0.5 - b)}{a}$$

Where IC_{50} = half maximal inhibitory concentration

RESULTS AND DISCUSSION

Percentage Yield of *S. singueana* Leaves in Different Solvent Extractions

As shown in Table 1, the highest yield was recorded in ethanol extract (1.66 g/100 g), followed by ethyl acetate extract (1.27 g), chloroform (0.54 g), and petroleum ether (0.50 g) in *S. singueana.*

Qualitative Phytochemical Analysis of *S. singueana*

As showed in Table 2, the findings of the present study of phytochemical screening of *S. singueana* leaves shown the presence of flavonoids, steroids, amino acids and proteins, carbohydrates, and cardioglycosides a tannin and phenolic compounds in ethyl acetate and ethanolic extracts investigated. Alkaloids and flavonoids were absent in petroleum ether and chloroform extracts. Among the four extracts, ethanolic extract was found to be rich in all the phytoconstituents. The result agrees with the view of Rashmi and Rajkumar,[11] who found that methanolic extract was more active and shown almost all tested phytochemicals in Lichen samples.

Table 1: The percentage yield of *S. singueana* in different solvents

Solvent	% yield mg/100 g
Petroleum ether	0.50
Chloroform	0.54
Ethyl acetate	1.27
Ethanol	1.66

S. singueana: Senna singueana

Quantitative *In Vitro* Antioxidant Assays

The radical scavenging activities of ethanolic leaf extract of *S. singueana* were probed against a series of free radicals such as DPPH radical, hydroxyl radical, NO radical, superoxide radicals, and reducing power.

DPPH radical scavenging activity

DPPH is relatively stable nitrogen-centered free radical. The reduction capability of DPPH is determined by its decreased absorbance at 517 nm as induced by natural antioxidants. The antioxidant activity (DPPH) of ethanolic leaf extract of *S. singueana* was shown in Figure 1, and the percentage of free radical (DPPH) was ranged from 30% to 69%. The IC_{50} value of ethanolic leaf extract of *S. singueana* was 92 µg/ml when compared to the standard ascorbic acid 44 µg/ml. This result was similar to the methanolic extract and ethyl acetate extract of *Adenium obesum* which scavenged DPPH radicals at a concentration of 80.61 ± 0.56 µg/ml and 82.56 ± 0.37 µg/ml, respectively.[12]

Reducing power

The reducing power capacity of compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been given to various mechanisms

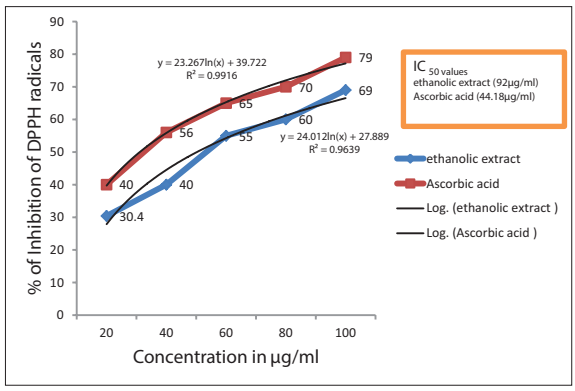

Figure 1: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity ethanolic leaf extract of *Senna singueana* compared to that of standard ascorbic acid. Each value is expressed as the mean ± standard deviation (*n*=3)

Table 2: Qualitative phytochemical analysis of different organic solvent crude extracts of *S. singueana* leaves

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	−	−	−	−
Flavonoids	−	−	+	+
Tannins	−	−	+	+
Glycosides	−	−	+	+
Terpenoids	−	−	−	+
Saponins	−	−	−	−
Steroids	+	−	+	+
Carbohydrates	+	+	+	+
Fixed oils and fats	+	−	−	−
Amino acids and proteins	+	+	+	+

+: Present, −: Absent. *S. singueana: Senna singueana*

such as prevention of chain initiation, binding of transition, metal ion catalysts, breakdown of peroxides, and prevention of continued hydrogen abstraction. The results show that there was an increase in reducing power of the plant extract as the extract concentration increases. Figure 2 explains the reducing power potentials of the ethanolic leaf extract of *S. singueana* in comparison with a standard ascorbic acid at 700 nm. Our results are correlated with increasing the concentration of water, ethanol, and aqueous ethanol extract of *Aerva Lanata* having reducing power activity reported by Ragavendran *et al*.[13]

Hydroxyl radical scavenging activity

As shown in Figure 3, results represent that the hydroxyl radical scavenging ability in both the cases was concentration dependent and was found to be maximum at a concentration of 100 µg/ml with inhibition of 69.44% with IC_{50} value of 57 µg/ml in case of plant extract and 79% in case of standard, with IC_{50} value of 46 µg/ml, confirming there by the significant antioxidant potential of *S. singueana* leaf extract. Kumari *et al.*[14] reported that aqueous leaves extract of *Anethum graveolens* shown hydroxyl radical scavenging activity in concentration-dependent manner.

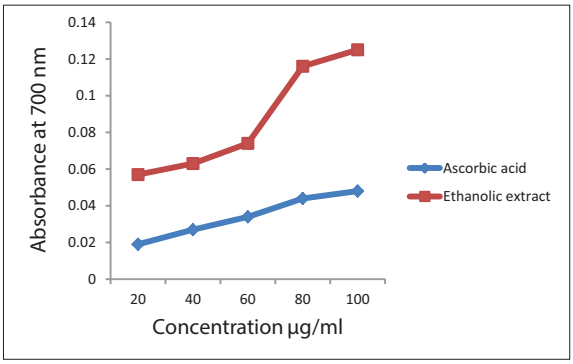

Figure 2: Reducing power activity of ethanolic extract of *Senna singueana* compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (*n*=3)

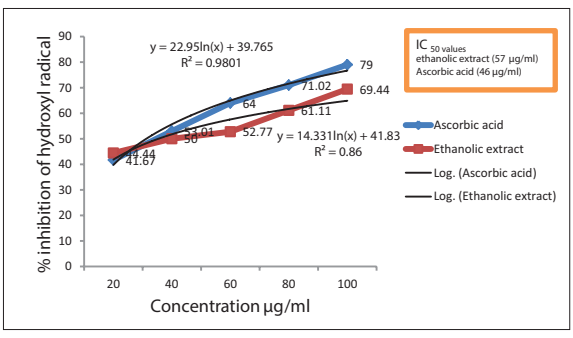

Figure 3: Hydroxyl radical scavenging activity of ethanolic extract of *S. singueana* compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (*n*=3)

NO radical scavenging activity of S. singueana

As shown in Figure 4, highest scavenging activity on NO radical is 61% for extract at the concentration of 100 µg/ml, with IC_{50} value of 113 µg/ml, where ascorbic acid exhibited 85.39% IC_{50} value of 23.23 µg/ml. NO is an important chemical mediator generated by endothelial cells, macrophages, and neurons, and complicated in the regulation of various physiological processes. However, excess concentration of NO is associated with several diseases, oxygen reacts with the excess NO to generate nitrite and peroxynitrite anions, which act as free radicals.[15] NO inhibitors have been shown to have beneficial effects on some parts of the inflammation and tissue changes seen in models of inflammatory bowel disease and diabetes mellitus.[16]

Superoxide radical scavenging activity

As shown in Figure 5, at a concentration of 100 µg/ml, the scavenging capacities for *S. singueana* extract and ascorbic acid were 53.33% and 73.33% with IC_{50} value of 191 µg/ml and 51 µg/ml, respectively. Superoxide is the first reduction product of molecular oxygen, a highly toxic radical, and the most abundantly produced in all

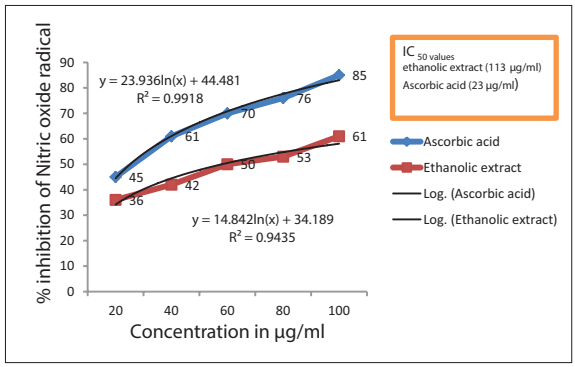

Figure 4: Nitric oxide scavenging activity of ethanolic extract of *Senna singueana* compared to that of standard ascorbic acid. Each value is expressed as the mean ± standard deviation (*n*=3)

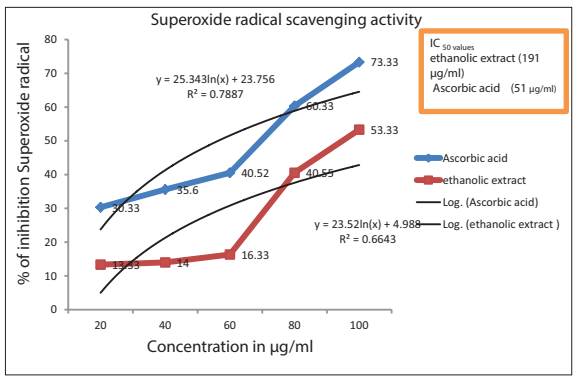

Figure 5: Superoxide radical scavenging activity of ethanolic extract of *Senna singueana* compared to that of standard ascorbic acid. Each value is expressed as the mean ± standard deviation (*n*=3)

aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules, and leads to unfavorable changes of biomolecules leads to oxidative stress-related disease.[17] The results of Sakthidevi and Mohan[18] clearly indicated that *Dioscorea alata* leaf extracts have a noticeable effect as a scavenging superoxide radical because of their higher total phenolic and flavonoid contents.

CONCLUSION

The results obtained in the present study have shown that the ethanolic leaf extract of *S. singueana* has a high content of bioactive secondary metabolites such as flavonoids, terpenoids, steroids, polyphenolic, alkaloids, tannins, and cardio glycosides and shown potential antioxidant activity. The ethanolic leaf extract of *S. singueana* was found to be an effective free radical scavenging activity on OH>DPPH>NO>O_2, respectively, compared to that of ascorbic acid and also possessed a good reducing power. This antioxidant activities of *S. singueana* provide a scientific knowledge to treat the oxidative stress-induced ailments and alternative for synthetic antioxidant drugs.

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