Comparative Study of Antioxidant Activity of Flower of *Aloe vera* and Leaf Extract of *Aloe ferox*

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Abstract: Great attention has been focused on medicinal plants and phytoconstituents due to their free radicals scavenging tendency. The present study investigates the comparative antioxidant ability of aqueous extracts of flower of *Aloe vera* and leaf of *Aloe ferox*. Antioxidant activity was assessed by using the DPPH, FRAP and $ABTS^+$ free-radical method. The IC_{50} values of aqueous extracts of flower of the *A. vera* and leaf of *A. ferox* in DPPH assay were 406.04 and 517.01 µg/ml, in $ABTS^+$ radical scavenging assay were 44.72 and 173.05 µg/ml, EC_1 in FRAP assay were 0.28 and 0.51 (mg/ml) respectively. Results of the present study showed that antioxidant capacity of whole leaf of *Aloe ferox* Mill was significantly higher than *Aloe vera* flowers.

Keywords: *Aloe vera*, *Aloe ferox*, Antioxidant activity, DPPH, phytoconstituents.

1. INTRODUCTION

Free radicals are chemical substances having one or more than one unpaired electrons formed as a result of various biochemical reactions in the cells [1, 2]. These biochemical reactions may be non-enzymatic or enzymatic [3].

The disturbance of balance of production and neutralization of pro-oxidant during metabolic processes [4, 5] in the body generates free radicals. A few examples of free radicals are the hydroxyl radical, hydrogen peroxide radical, singlet oxygen and superoxide anions. Such radicals are a source of variety for adverse effects like DNA mutation, protein damage, aging, cardiovascular diseases, carcinogenesis, and neuro-degenerative disorders. Free radicals are of concern as a result of exogenous factors [2, 6, 7], continuous exposure to chemicals, contaminants and oxidative stress and could be overcome by quenching the outcome of catalytic actions [5, 7].

Artificial drugs like rutin and butylated hydroxyl toluene, and butylated hydroxyl anisole are generally employed for anti-oxidant purposes. However, such drugs have adverse side effects like inflammation, damage to cells, atherosclerosis in mammals [2, 8] and toxicity. So, due to these problems, there is a developing worldwide tendency toward utilization of natural therapeutic antioxidant agents present in medicinal herbs and plants.

Recently investigators noticed that some herbal components such as polyphenols, flavonoids and terpenes have antioxidant activities [9]. According to the antioxidant role of these elements, researchers have more tendency to find natural antioxidants with high activity and low cytotoxicity [10].

Aloe ferox is commonly found in Asia, Lesotho, and South Africa. For many centuries, its leaf is utilized for constipation treatment [11]. Now it is famous due to its laxative, antiseptic, moisturizing, anti-inflammatory and cleansing properties. Traditionally, *Aloe ferox* is employed in the treatment of various health issues like gastrointestinal disorder, skin cancer, burns [12, 13] and inflammation. The gel of the leaf has been broadly recognized for significant laxative [14], wound healing [12], anti-inflammatory, cure of sexually transmitted disorders [15], gastrointestinal parasite control , immunostimulant, antitumor, antidiabetic [16], antimicrobial and antioxidant activities.

Aloe vera belongs to the *Liliaceae* family and is cultivated in many countries extensively in hot and dry weather conditions [17]. It has many certain bioactive compounds which are polysaccharides, glycoproteins, aloein, amino acids, proteins, , vitamins (A, B12, E, B, Choline), calcium, essential fatty acids, and anthracine [18] in its different parts. It has been documented to have many pharmacologic benefits including anti-inflammatory, immunomodulatory, anti-septic, antifungal, hypoglycemic or antidiabetic effects, anticancer, gastroprotective, increase in cardiovascular health, improvement of function of liver and also effective in skin disease treatment, burn-healing, wound healing and hypersensitivity health issues [17].

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2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Collection of Plant Materials and Preparation of Extracts

Fresh and full-grown leaves of *A. ferox* and flowers of *Aloe vera* were taken and cleaned to remove dust and dirt. The leaves and flowers were dried at 40°C in an oven to achieve constant weight and crushed to a uniform powder of suitable particle size. A suitable amount (200 g) of this powder material was extracted individually in methanol and distilled water on an orbital shaker (Stuart Scientific Incubator, SI 50, UK) for a period of about 48 hours. After filtration, the aqueous filtrate was frozen at a temperature −40°C and then dried for about 48 hours with the help of a freeze dryer resulting in a 31.2 g yield. Then extracts of desired concentrations were again constituted with selected respective solvents.

2.2. Chemicals Used

All analytical grade chemicals were used in this study. Some of these were 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid),1,1diphenyl-2--picrylhydrazyl, ferric chloride ($FeCl_3$), rutin , butylated hydroxyl toluene, Folin-Ciocalteu reagent, phosphate buffer , sodium carbonate and potassium ferricyanide.

2.3. Antioxidant Analysis Methods

The antioxidant activities of the flower of *A. vera* and the leaf extracts of *A. ferox* were evaluated using ABTS, DPPH, reducing power, nitric oxide, lipid peroxidation and hydrogen peroxide.

2.4. Ferric Reducing Ability of the Plant Extracts

The ability to reduce ferric to ferrous of the selected plant extracts was assessed with Aiyegoro [19] and Okoh methods. 2.6 ml of 0.2 M concentration of phosphate buffer having pH 6.6 was mixed with 2.55 ml potassium ferricyanide solution (1% w/v). In this mixture 1 ml of an extract and 1 ml of 0.026-0.5 mg/ml standards was added in distilled water. The resultant mixture was placed in an incubator for about 20 minutes at 51°C, then 2.5 ml of 10% w/v solution of TCA was added, then centrifuged at a speed of 3000 rpm about 10 minutes. Then 2.6 ml of the supernatant was added to a 0.6 ml solution of 0.1 % $FeCl_3$ and 2.6 ml of distilled water. Then the absorbance was measured at 700 nm parallel to a blank. An increase in absorbance of the resultant mixture proved high reducing ability of the plant extract.

2.5. Diphenylpicrylehydrazyl Assay

The prescribed method by Shahidi and Liyana-Pathiana [20] was adopted for the assessment of scavenging ability of 1,1-diphenyl-2-picrylhydrazyl radical. 1 ml of 0.136 mM DPPH solution was prepared in methyl alcohol and then blended with 1.0 ml of 0.025 to 0.5 mg/ml water extract. The reaction blend was vortexed thoroughly and then left in the dark at ambient temperature for a period of 30 minutes. Then its absorbance was measured with the help of spectrophotometer at wavelength of 517 nm. Then scavenging capability of the plant extracts was calculated by the following equation:

DPPH percent scavenging ability = [(Abs controlled – Abs of sample)]/(Abs controlled)] × 100,

where Abs controlled is absorbance of DPPH in methyl alcohol and Abs of sample is the absorbance of Diphenylpicrylehydrazyl radical in sample or standard.

2.6. ABTS Radical Scavenging Activity

The developed method by Adedapo [1] was followed for ABTS activity of the extract of plant. A working solution was made by mixing 7.1 mM ABTS solution and 2.42 mM solution of potassium persulfate in the same amounts and reacted in night for 12 h at ambient temperature. This solution was again diluted by addition of 1 ml of ABTS and extract solution in 60 ml of methyl alcohol and after 7 minutes absorbance was noted at 734 nm. For % inhibition calculation the following equation was used.

% inhibition = [(Abs controlled - Abs of sample)]/(Abs controlled)] × 100.

3. RESULTS

3.1. Antioxidant Studies of the Extracts

The extracts of *leaf of A. ferox* and flower of *A. vera* in different solvents showed good antioxidant activity in three antioxidant activity models assessed in this study.

3.1.1. Ferric Reducing Ability

The ability of the selected plant extracts to convert Fe^{3+} to Fe^{2+} by mean of electron transfer is a sign of good antioxidant potential. The reduction power of all solvent extracts of plant in comparison with butylated hydroxyl toluene is given in Table **1** and shown in Figure **1**.

Table 1: Ferric Reducing Power of Antioxidants

Concentration (mg/ml)	Ferric Reducing Power of Antioxidants					
	Vitamin C	BHT	Methanol Extract of *A. ferox* Leaf	Methanol Extract of *A. vera* Flower	Aqueous Extract of *A. ferox* Leaf	Aqueous Extract of *A. vera* Flower
0.025	0.22	0.21	0.39	0.15	0.13	0.04
0.05	0.5	0.37	0.40	0.17	0.15	0.07
1	0.91	0.75	0.41	0.18	0.16	0.9
0.2	1.72	1.18	0.43	0.22	0.19	0.12
0.5	2.65	1.98	1.21	0.68	0.63	0.36

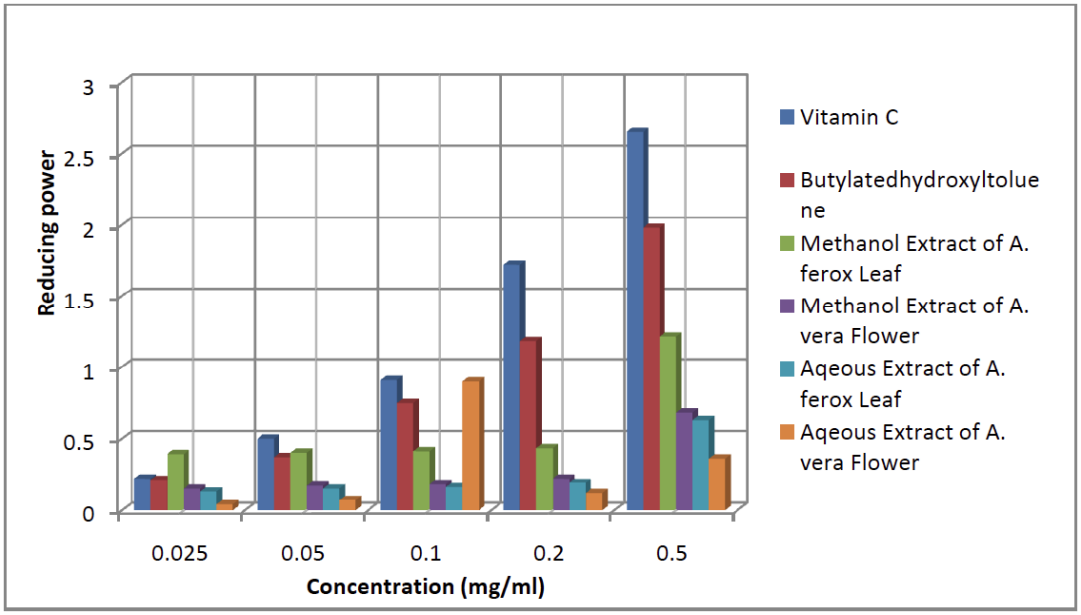

Figure 1: Ferric Reducing Power of Antioxidants.

The experimental change of yellow color of the prepared $FeCl_3$ solution to different green and blue colors, depending on actual concentration, is a sign of an active antioxidant agent. As compared to reference drugs reduction power order is: vitamin C > butylated hydroxytoluene > methyl alcohol > aqueous extract of leaf of *A. vera* > aqueous extract of flower of *A. vera*. At a concentration of 0.5 mg/ml, the absorbance of butylated hydroxytoluene and vitamin C were similar with methyl alcohol extracts.

3.1.2. Diphenylpicrylehydrazyl Assay

The Diphenylpicrylehydrazyl Assay of the leaf extracts of *A. ferox* and flower of *A. vera* was concentration dependent as shown in Table **2** and Figure **2**.

The order of percentage inhibition of Diphenylpicrylehydrazyl by the different extracts of plant and the reference drugs was found in decreasing order: butylated hydroxytoluene > rutin > methanol >aqueous extract of leaf of *A. ferox* > aqueous extract of flower of *A. vera*. The % inhibition shown by Butylated Hydroxyl toluene and rutin was notably different from the extracts, although the aqueous extract had minimum inhibition, but not considerably different from the other prepared extracts. The IC_{50} value of flower of *A. vera* was 0.288 mg/ml whereas methyl alcohol extract was 0.0861 mg/ml and that of aqueous extract leaf of *A. ferox* was 0.518 mg/ml.

3.1.3. ABTS Assay

The result of ABTS assay by the different solvent extracts of flower of *A. vera* and leaf of *A. ferox* is given in Table **3** and shown in Figure **3**.

It was clear that this activity exhibited by different solvent extracts of plant material was dependent on

Table 2: % Inhibition of DPPH Radical by Antioxidants

Concentration (mg/ml)	% Inhibition of DPPH Radical by Antioxidants					
	Rutin	BHT	Methanol Extract of *A. ferox* Leaf	Methanol Extract of *A. vera* Flower	Aqueous Extract of *A. ferox* Leaf	Aqueous Extract of *A. vera* Flower
0.025	77.5	81	18	8.86	11	3.75
0.05	80	90	36.5	18.3	18	10
1	79.8	95.5	58.4	37.7	36.4	18.2
0.2	83.8	97.5	81.3	75.1	74.2	41.7
0.5	96.3	98.8	82.7	79.8	81.2	76

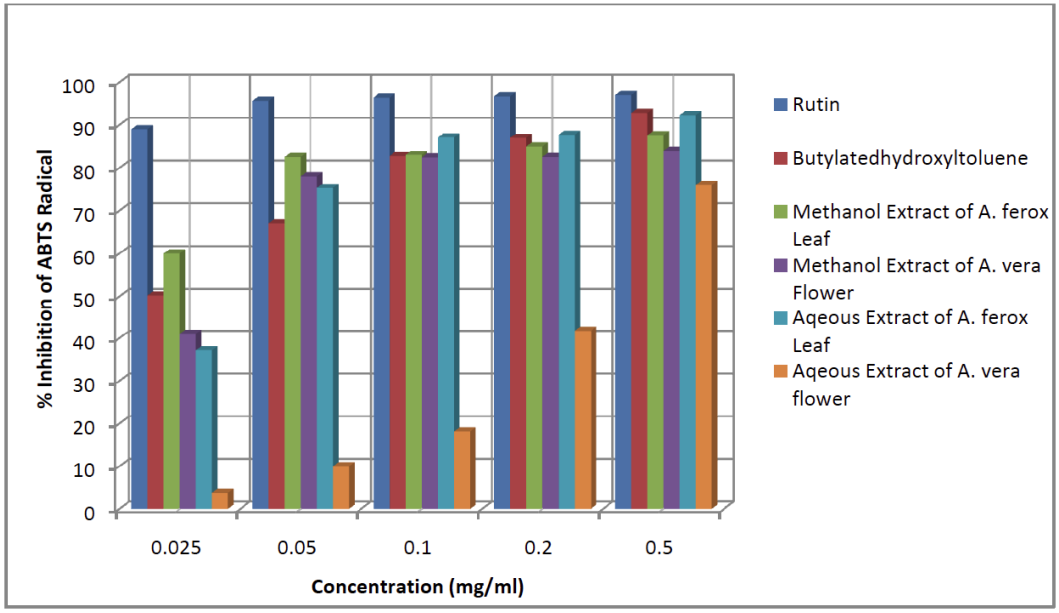

Figure 2: % Inhibition of DPPH Radical by Antioxidants.

Table 3: % Inhibition of ABTS Radical by Antioxidants

Concentration (mg/ml)	% Inhibition of ABTS Radical by Antioxidants					
	Rutin	BHT	Methanol Extract of *A. ferox* Leaf	Methanol Extract of *A. vera* Flower	Aqueous Extract of *A. ferox* Leaf	Aqueous Extract of *A. vera* Flower
0.025	89	50.2	60	41	37.2	12
0.05	95.7	67	82.5	78	75.3	31
1	96.4	82.4	83	82.4	87.1	55.7
0.2	97.6	87	85	82.5	87.7	77.4
0.5	97.1	92.8	87.6	84	92.3	79.5

concentration and compared satisfactorily well with the standard rutin and butylated hydroxyl toluene. The order of this activity was in decreasing order: rutin > methyl alcohol > butylated hydroxyl toluene > aqueous extract of leaf of *A. ferox* > aqueous extract of flower of *A. vera*. The extract in methanol compared satisfactorily well with Butylatedhydroxyltoluene (BHT) (Figure **3**). Different solvents at different concentrations showed a considerable difference. The IC_{50} values obtained by butylated hydroxyl toluene and methyl

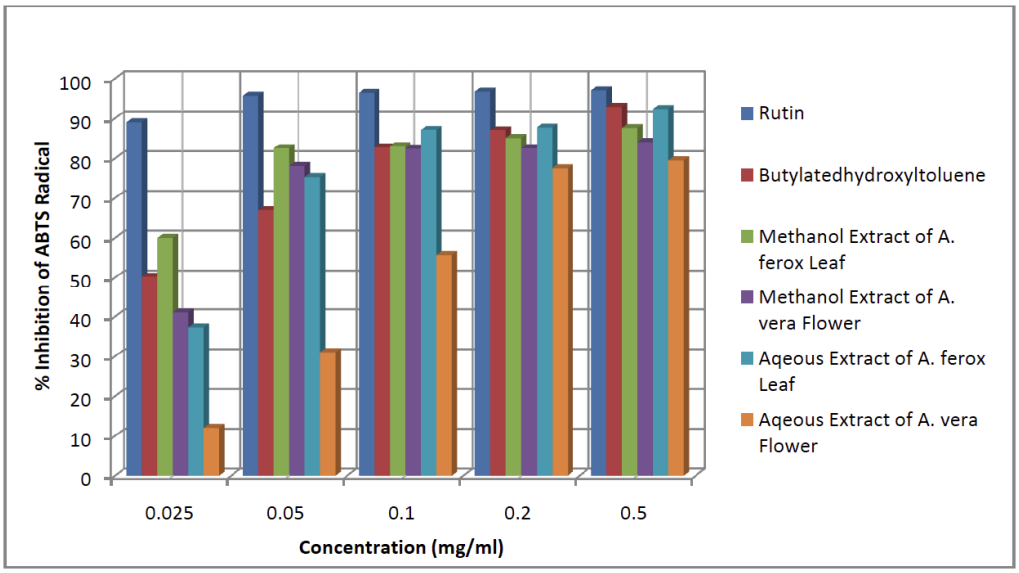

Figure 3: % Inhibition of ABTS Radical by Antioxidants.

alcohol extract were 0.024 and 0.02 mg/ml, respectively, followed by aqueous extract of leaf of *A. ferox* at 0.173 mg/ml, respectively. On the other hand aqueous extract of flower of *A. vera* exhibited the least activity (0.04472) as compared to other solvent extracts.

4. DISCUSSION

The capacity of substances to behave as antioxidant agents depends on their reduction potential for reduction of reactive oxygen entities by the donation of a hydrogen atom [21] generally due to the presence of reducing agent. The conversion of Fe^{3+} to Fe^{2+} was done by extracts of plant. *A. ferox* leaf extracts showed the reductive potentials in comparison with reference substances, butylated hydroxyl toluene and ascorbic acid. On the other hand flower of *A.Vera* has minimum reducing potential. It was also found that the reduction potential is concentration dependent and was comparable with butylated hydroxyl toluene and ascorbic acid.

The Diphenylpicrylehydrazyl is a fairly stable free radical scavenger that changes the single electron to paired electrons by the donation of a hydrogen proton. In this study it was seen that the scavenging ability of the DPPH radical by plant extracts at higher concentrations in all of the solvent extracts might be imply that the plant extracts potentially contain chemical substances that have the ability to donate protons to deactivate radicals. The method proved that the effectiveness of extracts is dependent on concentration. Plant extracts showed weak activity in the DPPH radical scavenging assay that might be due to the low level of flavonoid contents in the extracts of the plant.

The scavenging ability of the DPPH radical in different solvent systems was found to be lower than the scavenging ability of $ABTS^{+}$. This scavenging ability of the $ABTS^{+}$ radical by the extracts might be implied that the extracts can be useful for treatment of free radical related health disorders, particularly at higher concentrations.

5. CONCLUSION

The study presented shows the free radical scavenging activity of flower of *A. vera* and the leaf of *A. ferox* in aqueous and methanol extracts. This activity was higher in methanol extracts as compared to aqueous media, showing their antioxidant capacity. On the basis of these observed results further investigations to confirm the prophylactic effect of flower of *A. vera* and the leaf extracts of *A. ferox* for the treatment of health disorders caused by free radicals are suggested. Antioxidant activities mostly depend on the quantity of the phytochemicals in medicinal plants which, which are not very high both in the flower of *A. vera* and the leaf extracts of *A. ferox*, but can synergistically enhance their antioxidant activity. Thus these two species of the aloe family are strong antioxidant agents and thereby can provide credibility to the traditional usage of *A. ferox* extract as well as *A. vera extract*.

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