



TO EVALUATE THE ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF ALOE BARBADENSIS (EEAB), SALIX TETRASPERMA (EEST) AND TENACETUM PARTHENIUM (EETP) LEAVES.

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Abstract:

Aim: To evaluate the antioxidant activity of Ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves

Method: The crude drug was subjected to pharmacognostical evaluation and standardization for confirmation of its purity, then alcoholic extract of all these plants was subjected to qualitative phytochemical analysis and quantitatively it was identified for total phenolic and flavanoid content. In vitro anti oxidant activity was further evaluated by using DPPH scavenging assay, reducing power assay and Superoxide scavenging activity and IC₅₀ value was calculated.

Results: In this investigation, the in-vitro antioxidant effect of AB, TP and ST extract on DPPH radical scavenging activity of AB, TP and ST ethanol extract exhibited percent inhibition 83.39, 76.71 and 77.98% and its IC₅₀ value were found to be 25.97, 42.28, and 47.17µg/ml. Ascorbic acid was used as a reference compound which exhibited percent inhibition 82.61% and showed IC₅₀ value of 18.08 µg/ml. The AB, TP and ST ethanol extract displayed SOS activity which exhibited percent inhibition of 78.38, 6.17 and 71.67% and showed IC₅₀ value of 29.95, 47.87 and 54.17µg/ml. Similarly, for SOS activity, Ascorbic acid was used as a reference compound which exhibited percent inhibition 85.59% and showed IC₅₀ value of 14.6µg/ml.

Conclusion: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. The ethanolic extract of Aloe barbadensis showed strong reducing capacity as compared to other extract.

Keywords: invitro antioxidant assay, ethanolic extract, DPPH assay, Superoxide scavenging assay,

reducing power assay

Introduction

According to World Health Organization (WHO), there has been observed a tremendous increase in use of herbal medicines over the conventional drugs by 3-4 times. Herbal medicines are the oldest form of medicine known to mankind (1). Nature has granted medicine cabinet full of extraordinary plants and their natural products which forms the basis for many modern medicines. Also, with the increase in risk of side effects and adverse effects from the use of conventional drugs, it also leads our way towards the use of herbal medicines (2).

Aloe barbadensis Miller is a plant native to North and East Africa that has been with humans for over 5,000 years. The Aloe Vera plant has digestive, dermatological, culinary and beauty benefits. Based on this, Aloe has many opportunities for interesting research from different perspectives, including analysis of chemical properties, biochemistry involved in various activities, and their applications in pharmacology, as well as from horticultural and economic perspectives(3). The plant Salix tetrasperma is well considered for being utilized ethnobotanically for various purposes such as soil binder, fodder, baskets, sport articles, for making agricultural utensils, and treatment of various illnesses or disorders (4). Feverfew (Tanacetum parthenium L.) (Asteraceae) is a traditional medicinal herb used to treat migraines, fever, stomachache, rheumatoid arthritis, toothache, insect bites, infertility, and problems during pregnancy and childbirth (5).

Materials and Methods

Collection of Plant materials

Plant materials Aloe barbadensis and Salix tetrasperma was collected from Karond Bypass Road, Bhopal and Tenacetum parthenium was collected from Rutvik enterprises, Vasai West, Maharashtra. Special precaution was taken to collect healthy plant materials and foreign materials were avoided. Herbarium of plants were prepared and submitted to Department of Botany, Safia Science College, Bhopal, M.P.) for authentication. Plants were authenticated by Botanist Dr. Saba Naaz, Department of Botany, Safia Science College, Bhopal, M.P.) Plant authentication voucher number obtained was 173/Saif/Sci./College/Bpl for Aloe barbadensis, 174/Saif/Sci./College/Bpl for Salix tetrasperma and 175/Saif/Sci./College/Bpl for Tenacetum parthenium correspondingly.

Preparation of aqueous extracts

The extraction using the method of percolation in which dried plant sample was kept in contact with organic solvents such as ethanol for around 8-10 hours at 40-60°C temperature, in the thimble part of the Soxhlet apparatus. As soon as the extraction process completes, the extract was filtered, allowed to dry and percentage yield was calculated. Extracts were collected in air tight container till further use (6).

Pharmacognostical evaluation and Standardization of Crude Drug

1. Determination of Ash Value: Low-grade products, exhausted drugs and sandy or earthy matter are being identified using the ash value determination.

a) Total ash- Take around 4 gm of dried drug sample, place it in silica crucibles and allow it to ignite in muffle furnace at 600°C. After that take the crucibles out of the furnace and weight it after cooling it in the desiccator (7). Calculation for the percentage of total ash was carried out using the following formula

$$\text{Percentage of Total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

b) Acid insoluble ash- Add 45 ml of 1:5 hydrochloric acid in three 15 ml portions at a time to the ash-filled bowl, boil slowly for 5 minutes and strain. The insolubles were collected on ashless filter

paper (Whatman No. 41) and washed with distilled water until the residue was acid free. Transfer the filter paper containing the insoluble matter to the original container, dry and burn to constant weight (7). The Petri dish was weighed after cooling in a desiccator. Calculation for the percentage of acid insoluble ash was carried out using the following formula -

$$\text{Percentage of Acid insoluble ash} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of sample taken}} \times 100$$

2. Determination of Extractive Value: Plant materials contain water, minerals and organic compounds (primary and secondary metabolites). These compounds are extracted in different solvents at different concentrations. Extraction rate is the amount of components extracted with a solvent from an amount of plant material.

a) Alcohol soluble extractive- Weigh the amount of air-dried powder (3 g) in a glass stoppered glass. Add 100 ml of distilled ethanol (approximately 95%) and shake occasionally for 6 hours. After waiting for 18 hours, strain it (take care not to lose weight). Take the filtrate (25ml) without weighing 100ml into a bowl, evaporate to dryness in a water bath and then put in the oven at 105°C for 6 hours. After cooling the glass in the desiccator, it is weighed (8). Calculation for the percentage of alcohol soluble extractive was carried out using the following formula-

$$\text{Alcohol soluble extractive (\%)} = \frac{\text{Weight of the extract}}{25 \times \text{Weight of sample taken}} \times 100 \times 100$$

b) Water soluble extractive- Weigh the amount of air-dried powder (3 g) in a glass stoppered glass. Add 100 ml of distilled water and shake occasionally for 6 hours. After waiting for 18 hours, strain it (take care not to lose weight). Take the filtrate (25ml) without weighing 100ml into a bowl, evaporate to dryness in a water bath and then put in the oven at 105°C for 6 hours. After cooling the glass in the desiccator, it is weighed (8). Calculation for the percentage of alcohol soluble extractive was carried out using the following formula-

$$\text{Water soluble extractive (\%)} = \frac{\text{Weight of the extract}}{25 \times \text{Weight of sample taken}} \times 100 \times 100$$

3. Determination of Loss on Drying at 105°C: Weigh at least 2 g of air-dried powder in a bowl. It was then heated in an oven at 105°C for 5 hours. Weigh the Petri dish after cooling it in a desiccator (9). Calculation for the percentage of loss on drying was carried out using the following formula -

$$\text{Percentage of Loss on drying at 105°C} = \frac{\text{Loss in weight of the sample}}{\text{Weight of sample taken}} \times 100$$

4. Determination of pH Value: The pH of a 1% w/v aqueous solution can be defined as the logarithm of the corresponding hydrogen ion concentration expressed in grams per liter. The pH of the liquid can be determined with a glass electrode and a suitable pH meter (9).

Preliminary phytochemical screening

The extracts were tested for the screening of various Phytochemical contents such as carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using standard procedures (9,10).

Quantitative Estimation of Phytoconstituents

Total Phenolic Content

Total phenolic content of the extract was determined spectrometrically (11). Add 1 ml of Folin-Ciocalteu reagent (1:20) to 1 ml of sample (1000 µg/ml) and mix well with tannic acid (10-100 µg/ml). Add 4 ml of sodium carbonate to the mixture, make up the total volume to 10 ml with distilled water in the container and mix thoroughly. The mixture was allowed to stand at room temperature for

2 hours. The contents were centrifuged at 2000 rpm for 5 minutes and the absorbance of the supernatant was read at 760 nm. Standard curves were obtained using different concentrations of tannic acid. Results are expressed in milligrams of tannic acid equivalents (TAE) per gram of extract.

Total Flavonoids Content

Total flavonoid content was measured by aluminum chloride colorimetric assay (12). Add 1 ml of sample (1000 µg/ml) or quercetin standard solution (25-1000 µg/ml) to a 10 ml flask containing 4 ml of distilled water. 0.3 ml of 5% sodium nitrite was added to the above mixture. After 5 minutes, 0.3 ml of 10% aluminum chloride was added. Add 2 ml of 1 M sodium hydroxide over 6 minutes and bring the total volume to 10 ml with distilled water. Mix the solution well and measure the absorbance at 510 nm against a prepared reagent blank. The total flavonoid content of extracts is expressed as milligram equivalents of quercetin per gram of extract.

In-vitro Anti-oxidant Activity

1,1-Diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity

The free radical scavenging activity of extract was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) (13). About (3.5 ml) 0.1 mM DPPH solution was added to 0.5 ml of extracts sample solutions of various concentrations in methanol so that final volume made upto 4 ml. The mixture was allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Butylated Hydroxy Toluene was taken as standard antioxidant. Calculation for the percentage of free radical scavenging activity was carried out using the following formula -

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the test.

Reducing power assay

The reducing power of the extract was determined as per previously described method (14). Prepare different concentrations (20-100 µg/ml) in distilled water. Mix each concentration (0.5 ml) with phosphate buffer (1.5 ml, 0.2 M, pH 6) and potassium ferricyanide (1.5ml, 1%). Incubate the mixture at 50 °C for 20 minutes. One part (1.5 ml) of trichloroacetic acid (10%) is added to the mixture and then centrifuged at 3000 rpm for 10 minutes. The top layer of the solution (1.5 ml) is diluted with distilled water (1.5 ml). Finally, ferric chloride (300 µl, 0.1%) was added and centrifuged again at 3000 rpm for 5 minutes. And then measure the absorbance at 700 nm. An increase in the absorbance of the reaction mixture indicates an increase in attenuation. Ascorbic acid was used as the standard antioxidant. The experiment was carried out in triplicate.

Superoxide anion radical scavenging activity

Add 1 ml of NADH (468 µl, dissolved in 100 mM phosphate buffer in 1 ml of nitro blue tetrazolium (NBT) (100 µl of NBT, dissolved in 100 mM phosphate buffer, pH 7.4), pH 7.4) and to the solution add solutions of various concentration (20, 40, 60, 80 and 100 µg/ml). Start the reaction by adding 1 ml of phenazine methyl sulfate (PMS) (60 µl / 100 mM phosphate buffer, pH 7.4). Incubate the reaction mixture at 30 °C for 15 minutes. Measure the absorbance at 560 nm in a spectrophotometer. Incubate without adding sample (extract) as blank sample. Use ascorbic acid as a standard to compare different samples. The reduced absorbance of the reaction mixture indicates strong superoxide anion scavenging activity. (15).The percentage scavenging was calculated by using the formula shown below:

$$\% \text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the test.

Results and Discussion:

The results of physicochemical studies such as Ash content, extract value, moisture, pH value were all found to be in permissible limits according to Pharmacopeia standards (16) and previous reports. This indicated that the crude drug has successfully passed the standardization parameters which indicates that the crude drug is of sound quality (Table 1). The phytochemical screening reveals that ethanolic extract of all three plants proves to contain glycosides, alkaloids, terpenoids and phenolic compounds (Table 2). The quantitative estimation of phytoconstituents viz. total flavonoids and total phenolics revealed that ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves were found to be less amount of total flavonoids which is found to be 94.4 ± 0.32 , 57.5 ± 0.56 and 48.5 ± 0.41 quercetin equivalents mg/g of extracts, respectively while high amount of total phenolic content 111.25 ± 0.70 , 83.5 ± 0.24 and 67.75 ± 0.36 tannic acid equivalents mg/g of extracts, respectively.

In vitro antioxidant activity

1,1-Diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity

Ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and standard antioxidant, Ascorbic acid (20-100 µg/ml) induces DPPH free radical scavenging as indicated by concentration dependent decrease in the purple colour of the solution. The linear regression coefficient of ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and Ascorbic acid were found to be 0.992, 0.987, 0.990 and 0.989, respectively, suggesting that DPPH scavenging was concentration dependent. The IC₅₀ value of ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and Ascorbic acid were 25.97, 42.18, 47.17 and 18.08 µg/ml, respectively (Table 3).

Reducing power scavenging activity

Ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and standard antioxidant, Ascorbic acid (20-100 µg/ml) inhibited reducing power scavenging activity. The linear regression coefficient of ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and Ascorbic acid were found to be 0.982, 0.988, 0.998 and 0.997, respectively, suggesting that reducing power scavenging activity was concentration dependent (Figure 1).

Superoxide scavenging activity

Ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and standard antioxidant, Ascorbic acid (20-100 µg/ml) shows the Superoxide scavenging activity. The linear regression coefficient of ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and Ascorbic acid were 0.999, 0.988, 0.991 and 0.987, respectively, suggesting that Superoxide scavenging activity was concentration dependent. The IC₅₀ value of ethanolic extract of Aloe barbadensis (EEAB), Salix tetrasperma (EEST) and Tenacetum parthenium (EETP) leaves and Ascorbic acid were 29.95, 47.87, 54.17 and 14.08 µg/ml, respectively (Table 4).

Conclusion:

Antioxidants play an important role in protecting our body from diseases by reducing the oxidative damage caused by reactive oxygen species to cellular components. Recent studies have shown that plant-derived antioxidants with free radical scavenging properties may be effective in free radical-mediated diseases such as diabetes, cancer, neurodegenerative diseases, cardiovascular diseases,

aging, gastrointestinal diseases, arthritis and aging processes) have important clinical implications. Antioxidant activity of plant extracts was determined by various in vitro methods such as DPPH free radical scavenging activity, SOS activity and reducing potency test. A decrease in absorbance of the reaction mixture indicates greater activity, while an increase in absorbance of the reaction mixture indicates lower potency.

In this investigation, the in-vitro antioxidant effect of AB, TP and ST extract was evaluated. DPPH free radical scavenging activity of AB, TP and ST ethanolic extracts exhibited percent inhibition 83.39, 76.71 and 77.98% and its IC₅₀ value were found to be 25.97, 42.28, and 47.17 µg/ml. Ascorbic acid (a reference compound) exhibited percent inhibition 82.61% and showed IC₅₀ value of 18.08 µg/ml. The AB, TP and ST ethanol extract displayed SOS activity which exhibited percent inhibition of 78.38, 6.17 and 71.67% and showed IC₅₀ value of 29.95, 47.87 and 54.17 µg/ml. Similarly, for SOS activity, Ascorbic acid (a reference compound) exhibited percent inhibition 85.59% and showed IC₅₀ value of 14.6 µg/ml. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidants such as ascorbic acid were used for comparative evaluation for the in vitro antioxidant activity. Substances possessing the reducing power indicate that they are the energy sources that can reduce oxidative intermediates in lipid peroxidation, thus act as primary and secondary antioxidants. Compared with other extracts, the ethanolic extract of Aloe vera showed the most effective antioxidant compound with stronger reducing ability than other two extracts. Thus, we can conclude that ethanolic extracts shows the significant and reliable outcomes. Furthermore, animal study can reveal much more interesting facts supporting our current outcomes.

Conflict of interest: none

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Table-1: Pharmacognostical evaluation of plant sample

Parameters	Crude drug samples		
	<i>Aloe barbadensis</i>	<i>Tenacetum parthenium</i>	<i>Salix tetrasperma</i>
Total ash value	6.52	4.23	5.21
Loss on drying	12.56	10.52	10.87
Water soluble ash	3.89	3.16	2.96
Acid insoluble ash	2.15	2.11	1.54
Water extractive value	31.05	16.23	24.75
Alcoholic extractive value	14.58	12.50	12.14

All values are in percentage.

Table-2: Phytochemical analysis of ethanolic extracts

Experiment	<i>Ethanolic extract of Aloe barbadensis</i>	<i>Ethanolic extract of Tenacetum parthenium</i>	<i>Ethanolic extract of Salix tetrasperma</i>	
Test for Carbohydrates	Molisch's Test	+	-	+
	Fehling's Test	+	-	+
	Benedict's Test	+	-	+
	Bareford's Test	+	-	+
Test for Alkaloids	Mayer's Test	+	+	+
	Hager's Test	+	+	+
	Wagner's Test	+	+	+
	Dragendroff's Test	+	+	+
Test for Terpenoids	Salkowski Test	+	+	+
	Libermann-Burchard's Test	+	+	+
Test for Flavonoids	Lead Acetate Test	+	+	+
	Alkaline Reagent Test	+	+	+
	Shinoda Test	+	+	+
Test for tannins and phenols	FeCl ₃ Test	+	+	+
	Lead Acetate Test	+	+	+
	Gelatine Test	+	+	+
	Dilute Iodine Solution Test	+	+	+

Table-3: DPPH free radical scavenging activity

Concentration (µg/ml)	Percent (%) DPPH inhibition			
	Ascorbic acid	<i>Aloe barbadensis</i>	<i>Tenacetum parthenium</i>	<i>Salix tetrasperma</i>

20	49.72	46.87	38.16	37.00
40	59.05	57.00	48.76	47.49
60	69.35	65.37	61.56	54.70
80	76.48	74.55	67.42	64.91
100	82.61	83.39	76.71	77.98
IC ₅₀	21.18	25.97	42.25	57.18

Table-4: Superoxide scavenging activity

Concentration (µg/ml)	Percent (%)Superoxide scavenging activity			
	Ascorbic acid	<i>Aloe barbadensis</i>	<i>Tenacetum parthenium</i>	<i>Salix tetrasperma</i>
20	52.74	46.27	40.29	36.38
40	60.43	53.99	48.35	43.34
60	66.91	60.15	53.35	52.25
80	71.67	70.99	61.66	59.09
100	85.59	78.56	66.17	71.67
IC ₅₀	14.08	29.95	47.87	54.17

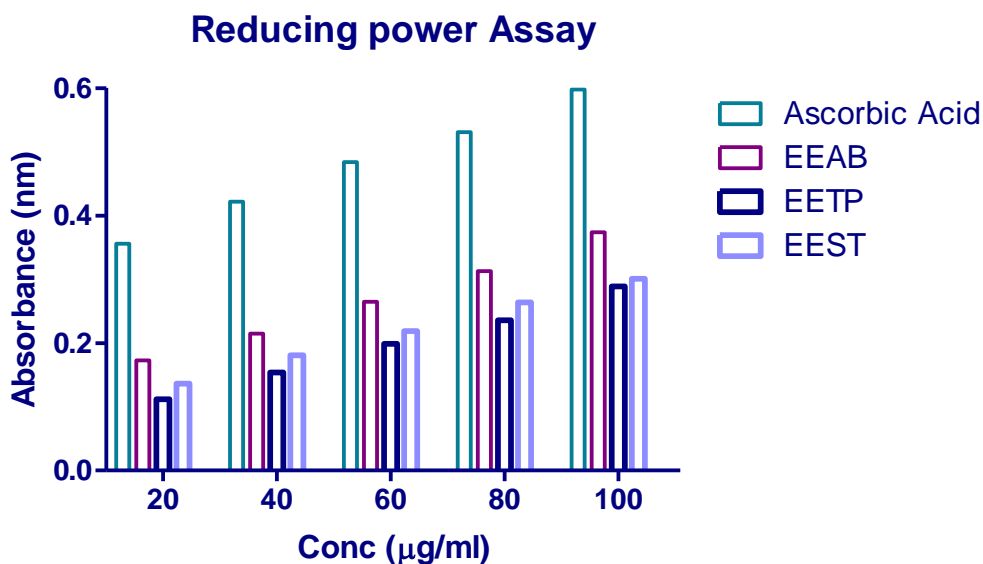


Figure-1: Reducing power scavenging activity