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Research Article

Antioxidant Potential of Indigenous Medicinal Plants of District Gujrat Pakistan

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Abstract

The work reported in this article was carried out to explore hidden antioxidant potential of some medicinal plants of District Gujrat, Pakistan. Crude methanolic extracts of *Cichorium intybus L, Malva sylvestris L,* and *Euphorbia milii L* were initially screened by DPPH on TLC assay for their antioxidant activity. Diphenylpicrylhydrayl (DPPH) free radical scavenging activity was also determined for all plants. To assess the role of plants in lipid oxidation, PV of refined bleached and deodorized (RBD) sunflower oil (SFO) at 80°C was monitored. BHT was used as standard antioxidant for comparison. Total phenolic contents (TPC) were also calculated. Cichorium intybus L was identified as the richest source of safe natural antioxidants.

Keywords: Antioxidants activity, TPC, PV, Medicinal plants

1.Introduction

Oxidative deterioration of vegetable oils and fats and foods containing oils and fats is a serious problem. Reactive aerobic species play key role in lipid oxidation [1] which is associated with cancer, aging and cardiovascular diseases. Free radicals are produced due to oxidation, which impart rancidity and odd flavors to lipids [2]. To counter the effect of these free radicals antioxidants are added which slow down the process of oxidation to increase shelf life of lipid containing foods. Butylated hydoxy anisole (BHA) and butylated hydroxyl toluene (BHT) are most popular synthetic antioxidants used for inhibition of oxidation but their toxicity has alarmed scientists and public [3, 4]. That is why researchers are very keen in safe antioxidants of natural origin. Many scientific reports are available in literature on antioxidant activities of medicinal plants because they are rich source of natural antioxidants. Phenolic compounds present in these plants tend to exhibit strong antioxidant potential [5]. Pakistan is very rich in availability of important medicinal plants. This piece of work was focused on investigation of some medicinal plants of Pakistan in context of exploration of natural antioxidant. *Cichorium intybus L, Malva sylvestris L, Euphorbia milii L* being very effective plants used in folk medicines were selected for current experimental study [6]. DPPH free radical scavenging activity of crude extracts of these plants was monitored in comparison to BHT. Lipid oxidation inhibition of crude plant extracts was observed by measuring changes in peroxide value (PV) of sunflower oil after 40 minutes storage at 80°C using BHT as standard. Total phenolic content (TPC) of all plants was also determined.

2. Materials and Methods

2.1. Chemicals and reagents

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) supplied by Walko Chemicals, Japan, BHT and Folin-Ciocalteu reagent by Sigma-Aldrich USA, prepared in the Laboratory were used. All the chemicals were of analytical grade.

2.2. Collection of plant samples

All the plants were collected from suburbs of City of Gujrat, Pakistan in 2012 and identified by comparing their botanical structures with those present in Botanical Herbarium of Government Zamindar College Gujrat, Pakistan.

2.3. Sample preparation

All plants as a whole were air dried in shade and powdered finely. The powdered materials were dipped in commercial methanol for 48h. Each extract was filtered separately and excess solvent was removed using a rotary evaporator equipped with a chiller and a temperature gauge. The crude extracts were resuspended in methanol to make 200 ppm solution. BHT was also dissolved in methanol for use as standard antioxidant.

2.4. DPPH on TLC assay

To assay antioxidant activity, dilutions of each plant extract were made in methanol (1:10) and 5 μl of each dilution was applied on TLC plates separately. TLC plates that constituted stationary phase were suspended in chromatographic tank containing a mixture of methanol and ethyl acetate in 1:1 composition for mobile phase. The TLC plates were subjected to TLC and removed from the tank, dried and sprayed with dilute solution of methanolic DPPH reagent (2mg/100mg) and allowed to stay for 35 minutes to check the bleaching of purple color of DPPH reagent to yellow spots[7].

2.5. Total phenolic contents (TPC)

To determine TPC of all extracts with Gallic acid as an internal standard, dilutions of extracts of each plant were made by homogenizing with 45ml of distilled water followed by addition of 1 ml of Folin-Ciocalteu reagent. After three minutes 3ml of 2% Na_2CO3 was added and resulting mixture was allowed to stand for 120min with shaking. Absorbance of the mixture was read at 760nm in UV-1700, Shimadzu, Japan spectrophotometer [8] and concentration of TPC in all extracts was expressed as μg of Gallic acid equivalent per gram of dry matter.

2.6. DPPH free radical scavenging

Dilutions of all extracts were made by adding 10ml of methanol to 10mg of each. To each diluted sample was added three ml of 6×10^{-5} M freshly prepared DPPH in methanol and 77μl of mixture was incubated for 15min at room temperature in dark and absorbance was read at 515nm [9]. BHT was used as control under same protocol. Percentage radical scavenging was calculated using the following formula.

$$\text{DPPH radical scavenging (\%)} = [(AB-AE)/AB] \times 100$$

Where AB = Absorbance of blank sample at t = 0; AE = Absorbance

of tested extracts after 15min incubation.

2.7. Peroxide value (PV) of sunflower oil

Refined, bleached and deodorized (RBD) sunflower oil (SFO) were collected; 200 ppm of each crude plant extract were added to 150 ml of refined, bleached and deodorized (RBD) sunflower oil (SFO); and homogenized for 20min at room temperature. SFO sample containing 200 ppm of BHT was used as standard. A blank SFO was also run under same conditions. All samples were heated in microwave oven at 80°C for 30min. PV was determined by recommended methods of AOCS [10]. Sunflower oil was selected due to its high level of unsaturation and extensive use in cooking. All the analyses were carried out in triplicate and data was statistically analyzed by general linear model ANOVA using Minitab16 software.

3. Results and discussion

All the crude plant extracts exhibited positive antioxidant activity bleaching purple color of DPPH reagent by yellow spots on TLC plates. The results of other assays explained the significant level of antioxidant potential. The antioxidant activity might be due to phenolic compounds present in plant extracts.

TPC results are reported in Table 1. Plant extract of *Cichorium intybus L* was found to be the richest source of naturally occurring phenolic compounds among all three plants. TPC of *Cichorium intybus L, Malva sylvestris L, Malva sylvestris L* were significantly different from each other, hence might be having key role in antioxidant activity proportional to their quantitative presence among plants.

Table 1. Total phenolic contents (TPC) in mg/g dried plant extract

Plant Extract	TPC
Cichorium intybus L	217.0 ± 2.00
Malva sylvestris L	175.0 ± 2.00
Euphorbia milii L	155.7 ± 2.00

± *indicates standard deviation of three experimental values.*

Free radical scavenging activity is directly related to concentration of phenolic compounds present in a plant [11]. Table 2 showed the percentage inhibition of DPPH radical by crude plant extracts.

Table 2. Percentage inhibition of DPPH radical by crude extracts of plants

Plant Extract	% inhibition of DPPH radical
Cichorium intybus L (Ci)	88.10
Malva sylvestris L (Ms)	77.30
Euphorbia milii L (Em)	68.70

Maximum antioxidant activity was exhibited by crude plant extract of *Cichorium intybus L* followed by *Malva sylvestris L* and *Euphorbia milii* L. Statistical analysis showed that all the values in Table 2 were significantly different. It clearly indicated the attribution of antioxidant activity to TPC of plants. The results were supported by findings of other studies and thus confirmed the role of biologically active compounds to encounter free radicals [12].

Peroxide value (PV) is a good indicator of oxidative deterioration of vegetable oils representing primary oxidation phenomenon. Many scientists reported the PV changes of vegetable oils with and without plant extracts to estimate the extent of oxidation [13-15]. Increase in PV was represented in Table 3.PV of SFO at start was 0.67±0.01. PV of all SFO samples increased from the starting value 0.67±0.01. Statistical analysis indicated that there was no significant difference in BHT and crude extract of *Cichorium intybus L* regarding retardation in increasing trend of PV.

Table 3. Relative Increase in PV (meq/kg) for Various SFO Samples at 80°C

PV of blank SFO	PV of BHT containing SFO	PV of Ci SFO	PV of Ms SFO	PV of Em SFO
3.78 ± 0.03	0.76 ± 0.01	0.79 ± 0.01	0.79 ± 0.01	1.27 ± 0.02

± *represents the standard deviation for triplicate values*

Once again high phenolic contents proved themselves strong antioxidants. Such a huge contribution towards antioxidant activity was amazing and provided good reason to replace toxic synthetic antioxidants by natural and safe substances of plant origin.

4. Conclusion

From the results and discussion emphasized, it may be concluded that Pakistani plants are rich source of natural antioxidants. Thus the work presented here may be further extended in two directions: (1) isolation and characterization of potent antioxidants from *Cichorium intybus L* for providing a strong basis for commercial exploitation; and (2) extension of spectrum of research to investigate the potential of antioxidant activity in all medicinal plants available in Pakistan.

Conflicts of interest

The author reports no conflicts of interest.

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