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Antioxidant Activity in the Leaves and Petals of *Calendula Officinalis* Linn.

Priyanka Pandey*, Bhagyashree Despande

Abstract

Aqueous extract of *Calendula officinalis* Linn. leaves and flowers has antioxidant action. The antioxidant activity was determined using the ferric thiocyanate and thiobarbituric acid techniques. The antioxidant activity of the aqueous extracts of petals was greater than that of leaves. According to the findings of this study, *C. officinalis* leaves and petals are a possible source of natural antioxidants.

Keywords: Antioxidant activity, *Calendula officinalis*, Ferric thiocyanate and Thiobarbituric acid methods

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Introduction

Reactive oxygen species (ROS), also known as active oxygen species, are diverse types of activated oxygen that include free radicals such as superoxide ions (O2-) and hydroxyl radicals (OH-), as well as non-free radical species like hydrogen peroxide (H_2O_2).[1,2] ROS may be produced in a variety of ways in live organisms, including by regular aerobic respiration, activated polymorphonuclear leukocytes and macrophages, and peroxisomes. Most of the oxidants produced by cells appear to come from these endogenous sources. Tobacco smoke, ionizing radiation, some pollutants, organic solvents, and pesticides are all exogenous sources of free radicals.[3-5] Food lipid peroxidation can be caused by free radicals, resulting in degradation.[6,7] Furthermore, ROS have been linked to more than a hundred disorders, including malaria, AIDS, heart disease, stroke, arteriosclerosis, diabetes, and cancer.[8-11] ROSs can cause tissue damage if they are created in excess. Tissue damage, on the other hand, might result in the production of ROS.[12] Nonetheless, all aerobic species, including humans, have antioxidant defenses and various damage removal and repair enzymes to remove or repair damaged molecules.[4,13-15] However, because this endogenous antioxidant system might be ineffective, dietary antioxidant supplementation is necessary.[11,16-17]

Butylated hydroxytoluene and butylated hydroxyanisole are two synthetic antioxidant chemicals often utilized in processed foods. It has been hypothesized, however, that these substances have potential adverse effects.[18,19] Furthermore, it has been hypothesized that dietary intake of antioxidant-rich foods and the occurrence of human illnesses are inversely related.[20]

As a result, it's critical to do study to identify natural antioxidant sources. *Calendula officinalis* Linn. (*Asteraceae*), sometimes known as Pot Marigold, is a medicinal plant used in traditional medicine to treat a variety of ailments. Calendula is a plant that is used in Ayurveda to cure fever and cancer.[21] It's employed because of its wide range of biological properties, including anti-inflammatory, anti-mutagenic, diuretic, and antispasmodic properties. It's also used to treat gastrointestinal, gynecological, ocular, and skin problems, as well as burns in rare situations. Many medicinal active ingredients, including as flavonoids, carotenoids, glycosides, and sterols, are abundant in the plant.[22] However, there is yet to be any evidence of this plant's antioxidant properties. Our primary goal is to identify viable natural antioxidant sources. The goal of this work, however, is to determine the antioxidant activity of *C. officinalis* Linn. leaf and petal extracts.

School of Sciences, MATS University, Raipur, Chhattisgarh, India

Corresponding Author: Priyanka Pandey, School of Sciences, MATS University, Raipur, Chhattisgarh, India. E-mail: Priyankapandey2907@gmail.com

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Materials and Methods

Plant Materials

The plant *C. officinalis* Linn. was taken from Raipur (Chhattisgarh), India's Local Herbal Garden, and was authenticated at Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, that was dried in shed and used for further work.

Extraction Preparation

In a blender, 400 g of dried leaves and petals were cut into small pieces, then extracted with 350 ml of hot water by stirring for 30 min, then filtered, concentrated, and dried.

Antioxidant Activity

Ferric thiocyanate (FTC) method

A solution of 5 mg of the material in 5 mL of 99.5% ethanol (final concentration 0.02%) was taken. In a vial with a screw top, 4.5 ml of 2.54% linoleic acid in 99% ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0), and 3.8 ml of water were combined and placed in an incubator at 400 C in the dark. 9.6 ml of 75% ethanol (v/v) and

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Table 1: Antioxidant activity of the aqueous extracts of leaves and petals of *Calendula officinalis* (FTC method)

Control	*Absorbance*								
	Day-1	*Day-2*	*Day-3*	*Day-4*	*Day-5*	*Day-6*	*Day-7*	*Day-8*	*Day-9*
	0	*0.387*	*0.394*	*0.434*	*0.42*	*0.463*	*0.49*	*0.584*	*0.463*
Vitamin C	0	0.038	0.047	0.049	0.064	0.10	0.198	0.122	0.259
Vitamin E	0	0.02	0.014	0.028	0.04	0.040	0.05	0.118	0.140
Aqueous leaves extract	0	0.02	0.02	0.039	0.040	0.057	0.07	0.084	0.15
Aqueous petals extract	0	0.004	0.006	0.008	0.012	0.015	0.028	0.045	0.063

Table 2: Antioxidant activity of the aqueous extracts of leaves and petals of *Calendula officinalis* (TBA method on the 10th day)

Extracts	*Absorbance*
Control	0.132
Vitamin C	0.076
Vitamin E	0.093
Aqueous extract of leaves	0.119
Aqueous extract of petals	0.113

0.1 ml of 30% ammonium thiocyanate were added to 0.1 ml of this combination. The addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture was done precisely 5 min later; (the absorbance of red color indicated the antioxidant activity) was measured at 500 nm every 24 h until the absorbance of the control reached maximum. The control and standard were submitted to the identical methods as the sample, with the exception that the control used just the solvent and the standard utilized 5 mg of Vitamins E and C instead of the sample's 5 mg.[23]

Thiobarbituric acid (TBA) method

The TBA technique is used to determine the degree of lipid peroxidation. Malonaldehyde binds to TBA at low pH and high temperature (100° C), forming a red complex that can be measured at 532 nm. To 3 ml of the mixtures containing the sample generated using the FTC technique, 3 ml of 30% trichloroacetic acid and 3 ml of 0.67% TBA solutions were added. After cooling to ambient temperature, the mixture was centrifuged at 3500 rpm for 25 min after being maintained in a water bath (100° C) for 15 min. The absorbance of the supernatant at 532 nm on the final day of the experiment was used to determine antioxidant activity.[24]

For both the FTC and TBA techniques, the percentage of antioxidant activity was determined using the formula.

$$\text{Percentage of antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Results

Table 1 shows the overall antioxidant activity induced by the extracts in terms of absorbance at 500 nm using the FTC technique. In the TBA technique, the control had the highest absorbance value (0.132), followed by extracts, aqueous extracts of leaves (0.119), and aqueous extract of bark (0.113), as shown in Table 2.

Discussion and Conclusion

Free radicals are potent antioxidants that include superoxide anions, hydroxyl radicals, and hydrogen peroxide. Free radicals are unguided missiles that ricochet about the body and assault healthy cells, ripping cell membranes, and causing genetic damage and abnormalities. They react with serum lipoprotein (LDL) and cause atheromatous plaques to develop, or they react with the lipids in cell membranes, causing polyunsaturated fatty acid peroxidation and the creation of more free radicals. As a result, antioxidants are required in several parts of the body, including the circulation system within cells, as well as the blood–brain barrier and the central nervous system. FTC and TBA techniques were used to test the antioxidant activities of *C. officinalis* Linn. leaves and flowers. The quantity of peroxide generated during the first stage of linoleic acid peroxidation was measured using the FTC technique. Peroxide interacts with ferrous chloride to generate ferric chloride, a crimson color. The concentration of peroxide decreases as the antioxidant activity rises in this approach. The absorbance levels in the control group increased from day 1 to day 8, then decreased on day 9 [Table 1]. This decrease is attributed to an increase in the unstable malonaldehyde molecules produced by linoleic acid oxidation. The last day's absorbance was used to calculate antioxidant activity in the TBA technique [Table 2]. It indicated the total peroxide values generated by linoleic acid oxidation. The lower the absorbance value, the lower the antioxidant content. In comparison to normal Vitamins C and E, the aqueous extract of leaves and petals has high antioxidant action based on absorption rates. In both the FTC and TBA methods, the aqueous extract of the petals showed lower absorbance, indicating that the petals have higher antioxidant activity than the leaves.

These extracts of *C. officinalis* may be effective in the treatment of malaria, AIDS, heart disease, stroke, arteriosclerosis, diabetes, and cancer due to their significant antioxidant activity.

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